ISSN 0001-6772
Published monthly for the Scandinavian Physiological Society

ACTA PHYSIOLOGICA SCANDINAVICA

VOLUME 100 NUMBER 1 May 1977

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APSCAX 100(1) 1–128 (1977)

Editorial office

Acta Physiologica Scandinavica
Karolinska Institutet
S 104 01 Stockholm

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From the Department of Physiology, University of Göteborg, Sweden

Effects of Volleys in Cortico-spinal Tract Fibres on Ventral Spino-cerebellar Tract Cells in the Cat

By

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Received 1 July 1976

Abstract

FU, T.-C., E. JANKOWSKA and R. TANAKA. *Effects of volleys in cortico-spinal tract fibres on ventral spino-cerebellar tract cells in the cat.* Acta physiol. scand. 1977. *100*. 1–13.

Both excitation and inhibition has been found in cells of origin of the ventral spino-cerebellar tract (VSCT) to be evoked by volleys in cortico-spinal fibres. The earliest EPSPs and IPSPs had features of disynaptically evoked postsynaptic potentials; these were, however, found only in a small proportion of cells and polysynaptic EPSPs and IPSPs were dominating. Postsynaptic potentials evoked in VSCT cells from primary afferents were effectively facilitated by cortico-spinal volleys. The cortico-spinal effects on VSCT cells may thus well be mediated by the same interneurones which mediate their excitation or inhibition from the periphery and which could evoke similar postsynaptic potentials in motoneurones. Generally all the observations are in keeping with the hypothesis (Lundberg 1971) that VSCT cells monitor transmission through interneurones interposed in various reflex paths to motoneurones.

According to the hypothesis recently put forward by Lundberg (1971) the ventral spino-cerebellar tract (VSCT) is primarily involved in monitoring transmission through spinal interneurones to motoneurones. Monosynaptic excitation and disynaptic inhibition from a given fibre system is often evoked in one and the same VSCT cell. If they are evoked via collaterals of fibres which terminate on inhibitory interneurones and via axon collaterals of these interneurones respectively, the VSCT cell may well compare the inhibitory output from the interneurones in question with their excitatory input. Such a comparison in respect to last order inhibitory interneurones of various reflex paths to motoneurones was considered as one of the main functions of VSCT cells. A comparison of the output from the last order inhibitory interneurones with their inhibitory input, of the output from the earlier order inhibitory interneurones with their excitatory or inhibitory input and of the excitatory and inhibitory inputs to motoneurones (or interneurones) from different fibre systems were other proposed functions.

Lundberg's hypothesis was easiest to test for those cases in which monosynaptic and disynaptic excitation and/or inhibition of VSCT cells was evoked in parallel with disynaptic

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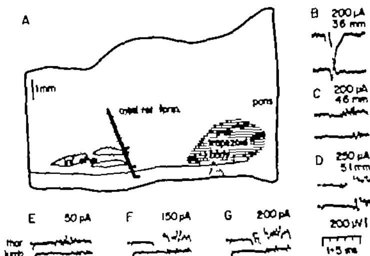


Fig. 1. Descending volleys evoked by stimulation of medullary pyramids. A shows reconstruction of an electrode track and of lesion of the pyramidal tract at level of the trapezoid body. The electrode was introduced 4 mm rostral to the obex with an angle of 30° from vertical. B–D and E–G, records of descending volleys from the surface of the lateral funiculus at low thoracic (upper traces) and mid-lumbar (lower traces) level in two different experiments. B–D records of descending volleys evoked by stimuli applied at approximately the same position of the electrode as in E–G and at positions 0.5 and 1.5 mm dorsal to it, as indicated by dots to the right of the electrode track in A. Strength of pyramidal stimulation is indicated above the records in µA.

Källström 1971) mounted over the exposed cortical area. Pyramids were stimulated either bipolarly through 2 electrodes in contact with their surface (introduced through hole in the base of the skull and fixed with plastic padding) or monopolarly through tungsten electrode introduced through the 4th ventricle. The strength of pyramidal stimulation was just maximal for descending volleys evoked by single shocks with latency typical for the fastest conducting cortico-spinal fibres and subthreshold for evoking descending volleys from more dorsally located structures (see Fig. 1).

Recording. Intracellular records were taken from VSCT cells with micro-electrodes filled with potassium citrate solution and tips broken to about 1.5 µm. Records from the surface of the spinal cord were taken with silver ball electrode in contact with the lateral funiculus in L5, close to the dorsal root entry zone and only few millimeters rostral to the level of penetration of the microelectrode. Single sweep or averaged records of postsynaptic potentials were taken. Averaging was done either with CAT 1000 or with Hewlett-Packard type 5480A.

Fig. 1 shows reconstruction of an electrode tract in para-sagittal plane about 0.7 mm from the midline. The electrode tip was positioned in the middle of the pyramid at level of the rostral border of the inferior olive. The descending volleys recorded from the surface of the lateral funiculus at thoracic and at lumbar level, as indicated, appeared then with stimulus strength of 20 µA and their amplitude reached the maximum with about 150 µA stimulus strength (*cf.* F and G). There were no indications for current spread to other descending systems with currents up to about 250 µA (D) but already 0.5 mm dorsally 200 µA evoked smaller responses in the corticospinal tract and current spread to other descending fibres (C). 1.0 mm more dorsally 200 µA were ineffective in exciting cortico-spinal fibres (*cf.* B and D) and instead of them activated some other most likely reticulo-spinal fibres with much higher conduction velocity.

Sample of analysed VSCT cells. From the point of view of their input VSCT cells constitute very non-uniform population of cells (Oscarsson 1957, Eccles, Lundberg and Oscarsson 1961, Lundberg and Weight 1971, Lindström 1973) but all their main subgroups were represented in the sample of cells on which the present analysis was done. The sample included 75 cells located in L4–L6 which were identified by their antidromic activation from the contralateral spinal half at Th 11–12, by their monosynaptic excitation from group I afferents and/or by their inhibition from the FRA (Eccles and Lundberg 1959). Monosynaptic excitation from group Ia and from group Ib afferents occurred in 44 and in 26 of the cells respectively.

excitation or inhibition of motoneurones. The postsynaptic potentials evoked in VSCT c from group Ia afferents (Lundberg and Weight 1971 Gustafsson and Lindström 1 Lindström 1973), from group Ib afferents (Lundberg and Weight 1971 Lindström a Schomburg 1974), from Deiters nucleus and medial longitudinal fasciculus (Baldissera a Roberts 1975 1976) and from the red nucleus (Baldissera and Bruggencate 1976) v subsequently found to be fully compatible with the proposed collateral connexions from t spinal reflex paths to motoneurones Previous studies on effects evoked in VSCT cells fro the corticospinal tract (Magni and Oscarsson 1961) led, on the other hand to two concl sions, one supporting and one apparently contradicting the validity of Lundberg's hyp thesis in respect to these effects. The conclusion supporting the hypothesis was that inhibiti evoked in VSCT cells from the cortico-spinal tract fibres is mediated by interneurones wh are activated also from the flexor reflex afferents inhibition of VSCT cells via these int neurones might thus well be parallel to inhibition of motoneurones. The conclusion variance with the hypothesis was that volleys in the pyramidal tract fibres evoke only inhib tion in the VSCT The latter conclusion was based on observations that of two effects stimulation of the postcruciate cortex, excitation and inhibition, the latter but not the form was abolished by lesions of the pyramidal tract Excitation evoked from the same cortic area as well as from other parts of the cortex was therefore attributed to extrapyramid systems. The possibility that the excitation was evoked in parallel via both the extrapyr midal and pyramidal systems was not however ruled out and it may get some support i additional observations of Magni and Oscarsson (1961) on the latency and time course facilitatory effects evoked from different cortical areas. Those induced from the postcrucia and the neighbouring precruciate cortex had shorter latency and earlier maximum than th facilitatory effects evoked from other areas. Different pathways may thus mediate excitati of VSCT cells from the cortex and the corticospinal tract might be one of them.

The aim of the present study was to verify if Lundberg's hypothesis can be applied al to cortico-spinal effects on VSCT cells and in particular to check if excitation of these ce is evoked from pyramidal tract fibres and might be mediated by interneurones of spin reflex paths similarly as their inhibition.

Methods

Preparation The experiments were perf rmed 11 cats under chloralose anesthesia (50–70 mg kg ntubation and dissection being done under ether The cats were paralyzed with Flaxedil nd artificial ventilated. Blood pressure and end tidal CO_2 level were mon tored continuously and kept t more than 9 mmHg and between 3 5 nd 4 0 respectively The dissection included (I) dissectio of a umber of musc and skin nerves of a hindlimb (see under Abbreviations), (I) laminectomy at L3–L6 spinal levels and sectio of L6, L7 and S1 ventral roots, the proximal ends of which were used f stim lation recording, (ii laminectomy at Th 12 Th 13 spinal levels, removal of the dorsal columns lo g about 2 segments, hemi section t the side contralateral t the dissected nerves, separation of the co tralateral pinal half in th caudal direction for stimulation and antidromic activation of VSCT cells, lesion of the ventral part of th ipsilateral spinal half and (i) exposure of the contralateral pericruciate cortex (i 6 cat which bod motor cortex d pyramids were stimulated) or pyramidotomy at a rostral medullary level (5 cats i which only pyramids were stimulated).

Stimulation Moto cortex was stimulated through glass microelectrodes f lled w th 2 M NaCl solution and tips broken to about 2µm, using 0.2 or 0.5 ms negative pulses up to 10–70 µA. The microelectrode were positioned close t pyramidal tract (PT) cells described previously (J k w ka Padel nd T naka 1975). Pulsations of the brain were prevented or diminished by use of cl...

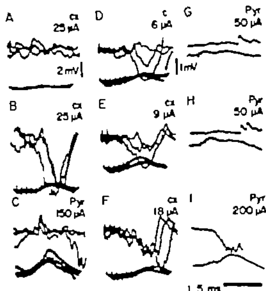


Fig. 3. EPSPs and IPSPs evoked by near threshold stimuli. Records in A–C, D–F and G–I are from 3 different VSCT cells. Note that the weakest stimuli (in A, D, G and H) evoked in them distinct EPSPs. With an increase in the number of shocks (B, I) or the intensity of current (E, F, I) the more and more pronounced IPSPs practically masked the EPSPs. C shows an EPSP evoked by pyramidal stimuli in the cell illustrated in A–C. Preparations with intact pyramids stimulated at surface (A–F) and with pyramids cut at rostral medullary level and stimulated with needle electrode (G–I).

in which the conduction velocity is considerably slower (see Jankowska *et al.* 1974, Wall and Werman 1976). The onset of EPSPs set up at longer distances from the soma is also delayed (see records of unitary monosynaptic EPSPs with different rise times in Mendell and Henneman 1971). However temporal facilitation of practically all short latency EPSPs evoked from the cortico-spinal tract, with an increase in the amplitude of the responses evoked by successive shocks in a train (Fig. 2) in a way typical for disynaptic responses (see Jankowska *et al.* 1974) speaks against their monosynaptic coupling. In addition, the latencies of the earliest EPSPs evoked by single shocks were within the same range as the shortest latencies of IPSPs observed under similar conditions (see below) and as latencies of clearly not monosynaptic EPSPs or IPSPs evoked only by a second or a third shock in a train (Fig. 7). Since in none of the 15 VSCT cells in which relatively early EPSPs were evoked by single stimuli could any positive evidence for their monosynaptic character be found, they were all classified as di- or polysynaptic.

For differentiation between the disynaptic and tri- or polysynaptic EPSPs the segmental latencies were of even less use and the only applicable criterion was the shock-locking (as in Fig. 2) which is regularly observed in the case of properly identified disynaptic, but not polysynaptic responses (see Jankowska *et al.* 1974). Shock-locking defines thus the above referred responses as disynaptic in contrast to such responses as shown in Fig. 4, 6 and 8 which are classified as polysynaptic. The EPSPs with the features of the disynaptic responses were of much smaller amplitude, usually not exceeding 0.5 mV, than mono- or disynaptic EPSPs evoked in VSCT cells from group I afferents (Eccles, Hubbard and Oscarsson 1961, Lundberg and Weight 1971, Lindström and Schomburg 1974) and from previously investigated descending systems (Baldissera and Roberts 1976, Baldissera and Bruggencate 1976). They were also smaller than EPSPs of cortical origin evoked polysynaptically. The major excitatory input from the pyramidal tract to the VSCT cells appears thus to be poly

Di- or trisynaptic EPSPs were evoked from cutaneous afferents (in 3 %). Disynaptic IPSPs were evoked from group Ia afferents (in 18 %), from group Ib or high threshold group I afferents (in 30 %) and from cutaneous afferents (in 7 %). In view of a large proportion of cells with monosynaptic excitation from group Ia afferents the sample was probably biased towards the spinal border cells (Lundberg and Weight 1970).

Abbreviations. Cortex, cx deep peroneal nerve including branches to tibialis anterior and to extensor digitorum longus, DP excitatory postsynaptic potential, EPSP flexor digitorum longus and hallucis nerve, FDL, flexor reflex afferents, FRA gastrocnemius-soleus nerve, G–S inhibitory postsynaptic potential, IPSP peroneus longus, tertius and brevis nerves, Per plantaris nerve, Pl postsynaptic potential PSP pyramids, Pyr suralis nerve, Sur superficial peroneal nerve, SP threshold, T tibial nerve, including cutaneous branches and branches to foot muscles, Tib quadriceps nerve, Q ventral roots, VR, ventral spino-cerebellar tract, VSCT

Results

I. *Excitation of VSCT cells from the cortico-spinal tract*

Cortico-spinal tract fibres have polysynaptic connexions with last order interneurones of segmental reflex paths to lumbar motoneurones in the cat except probably those which mediate the shortest latency (di- or trisynaptic) PSPs from cutaneous afferents and may be monosynaptically excited from the pyramidal tract (see Lundberg, Norrsell and Voorhoeve 1962, Fetz 1968 Hongo Jankowska and Lundberg 1969). Accordingly polysynaptic EPSPs or IPSPs are as a rule evoked in cat lumbar motoneurones by stimulation of the motor cortex or the medullary pyramids and those with features of disynaptic responses are observed only occasionally (Fig. 2 C, D). If excitation of VSCT cells following stimulation of cortico-spinal tract fibres is collateral to excitation of last order interneurones of segmental reflex paths to motoneurones (Lundberg 1971) it would thus be more likely poly- than monosynaptic. Direct coupling between cortico-spinal tract fibres and VSCT cells should on the other hand be found if these cells conveyed information on transmission through earliest order interneurones.

1 *Early excitation* The earliest EPSPs evoked in VSCT cells by single shock stimulation of the pyramidal tract had segmental latencies of 1.3 to 5 5 ms in respect to the earliest components of the descending volleys. Although such latencies make it rather unlikely they cannot by themselves disprove that these EPSPs were evoked monosynaptically if e they were evoked by some slower conducting fibres Fig. 1 shows that there may be more than 5 ms delay between the earliest and the latest components of the pyramidal descending volley Segmental latencies longer than 1 0 ms might likewise be compatible with monosynaptic transmission from fast conducting fibres with long and/or thin terminal collaterals

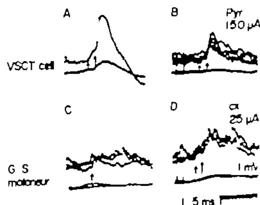


Fig. 2. Temporal facilitation of short latency EPSPs. Records in A, B and C, D are from a VSCT cell and from motoneurone respectively (upper traces) and from the surface of the spinal cord (lower traces). Averaged records are in A and superimposed single sweep records in B–D. Note larger amplitude of EPSPs evoked by the second stimulus and their time-locking with the stimuli. Records A and B are from the preparation illustrated in Fig. 1 A; those in C and D are from preparation with intact pyramids.

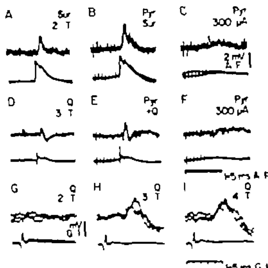


Fig. 5 Pyramidal facilitation of early EPSPs evoked from peripheral nerves. In A–C and D–I are records from 2 VSCT cells, in which EPSPs were evoked from cutaneous afferents (A) and from group II afferents (D). These were facilitated by preceding pyramidal stimulation (B, E) which by itself evoked only weak excitation (C, F). Records G–I show that the test EPSP was evoked only when the strength of Q stimulation was increased from that measured for group I afferents (G) to three times threshold (H) and that the amplitude of the conditioned response reached the amplitude of the EPSP evoked by somewhat stronger stimulation of Q nerve (cf. E and I).

Bruggencate and Lundberg (1971). It could, however, be shown that interneurones which mediated excitation of the same VSCT cells from primary afferents were excited by cortico-spinal tract fibres and could thus mediate excitation evoked from them as well. This is illustrated in Fig. 5 with facilitation of a relatively early excitation from cutaneous afferents from Sur and from group II afferents from Q nerve. Facilitation of somewhat later excitation evoked from group I afferents in FDL and from cutaneous afferents in Sur and Tib is shown in Fig. 6.

That EPSPs of cortical and of peripheral origin may be mediated by common interneurones is indicated also by records such as shown in Fig. 4. The configuration of EPSPs evoked in a number of VSCT cells by stimulation of the pyramidal tract showed namely great similarity to the configuration of EPSPs evoked from some of the dissected nerves, and a similar relation to the IPSPs accompanying them. In the cell illustrated in Fig. 4 A–F predominant IPSPs were evoked from all the nerves (exemplified in E and F) except Pl and Sur. Stimulation of the latter gave rise to an EPSP or to an EPSP followed by an IPSP which greatly resembled the responses evoked from the pyramidal tract by weak (A) or somewhat stronger (B) stimuli. In the cell illustrated in Fig. 4 G–J similar effects, EPSPs superimposed on IPSPs, were evoked from the pyramidal tract (G–H) and from Tib (I) and Sur (J), while stimulation of other nerves evoked IPSPs mixed with EPSPs to a much smaller extent. It is thus possible that the cortical effects were mediated in these two cells by interneurones interposed in relatively "private" reflex paths activated from Pl, Sur or Tib and not shared by other FRA. In other cells EPSPs evoked from the pyramidal tract resembled EPSPs evoked from a larger number of peripheral nerves. These EPSPs might thus have been mediated by interneurones intercalated in less specific FRA pathways.

II. *Inhibition of VSCT cells from the cortico-spinal tract*

1. *Early and late IPSPs.* Inhibition was a prevalent effect of volleys in the cortico-spinal tract fibres in nearly all the VSCT cells even if single shocks or short trains of weak stimuli

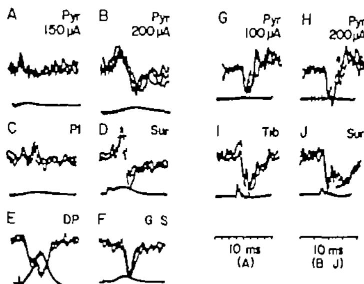


Fig. 4 A comparison of EPSPs evoked from the cortico-spinal tract and from primary afferent and G–J are from two different VSCT cells. Uppermost records (A B and G H) show effects of 2 d strengths of pyramidal stimulation. The remaining records show EPSPs and IPSPs evoked from di peripheral nerves. Note that EPSPs appeared as either a predominant or a first component in the f (A D) or as a later component in the second cell (G J), whether evoked by cortico-spinal or peri volleys. The similarity did not, however hold true for all the nerves, e.g. not for those i E, F for th cell.

synaptic. When estimating the size of these earliest EPSPs it must, however be taken account that they may have been masked by IPSPs which were evoked simultaneousl which followed them closely As illustrated in Fig. 3 the EPSPs were sometimes more dis when evoked by one or two shocks than by a train of shocks (cf A and B, and G H a and/or when the stimulus strength was low (cf D E and F I and G H and I) and a l limited number of cortico-spinal tract fibres was activated. Early EPSPs were seen in 1 excited from Ia afferents In 6 cells excited from Ia and Ib afferents, in 2 cells excited Ib afferents and in 6 cells only inhibited from the FRA

2. *Polysynaptic excitation.* Distinct polysynaptic excitation appeared on stimulatio the cortico-spinal tract in about two thirds of the VSCT cells. The EPSPs preceeded or c lapped the early components of the IPSPs in 32 out of 75 analysed cells as illustrate Fig. 4 A, B They appeared in addition during a late phase of the IPSPs in some of the s 32 cells, and in 20 other cells as shown in Fig. 3 E, F 4 G H and 7 C As a dominating e the polysynaptic excitation was found only in 7 cells (as in Fig. 4 A) However since relative amplitudes of the EPSPs and of the IPSPs depended on the stimulus strength Fig. 3 E and F and Fig. 4 A and B) and on the membrane potential of the cells, the percent of cells with the excitation dominating under certain testing condition might be somew higher

3 *Relation between EPSPs of pyramidal and of peripheral origin* Since the earliest EP evoked from the pyramidal tract fibres were of a small size and usually mixed with m more pronounced IPSPs it was difficult to find out if they were facilitated by condition stimulation of some peripheral nerves, and mediated by interneurones interposed in one the known reflex pathways according to the procedure of reverse conditioning of Baldisse

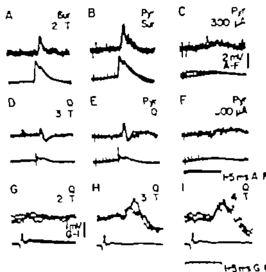


Fig. 5. Pyramidal facilitation of early EPSPs evoked from peripheral nerves. In A–C and in D–I are records from 2 VSCT cells, in which EPSPs were evoked from cutaneous afferents (A) and from group II afferents (D). These were facilitated by preceding pyramidal stimulation (B, E) which by itself evoked only weak excitation (C, F). Records in G–I show that the test EPSP was evoked only when the strength of Q stimulation was increased from that maximal for group I afferents (G) to three times threshold (H) and that the amplitude of the conditioned response reached the amplitude of the EPSP evoked by somewhat stronger stimulation of Q nerve (cf. E and I).

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That EPSPs of cortical and of peripheral origin may be mediated by common interneurones is indicated also by records such as shown in Fig. 4. The configuration of EPSPs evoked in a number of VSCT cells by stimulation of the pyramidal tract showed namely great similarity to the configuration of EPSPs evoked from some of the dissected nerves, and a similar relation to the IPSPs accompanying them. In the cell illustrated in Fig. 4 A–F predominant IPSPs were evoked from all the nerves (exemplified in E and F) except Pl and Sur. Stimulation of the latter gave rise to an EPSP or to an EPSP followed by an IPSP which greatly resembled the responses evoked from the pyramidal tract by weak (A) or somewhat stronger (B) stimuli. In the cell illustrated in Fig. 4 G–J similar effects, EPSPs superimposed on IPSPs, were evoked from the pyramidal tract (G–H) and from Tib (I) and Sur (J), while stimulation of other nerves evoked IPSPs mixed with EPSPs to a much smaller extent. It is thus possible that the cortical effects were mediated in these two cells by interneurones interposed in relatively "private" reflex paths activated from Pl, Sur or Tib and not shared by other FRA. In other cells EPSPs evoked from the pyramidal tract resembled EPSPs evoked from a larger number of peripheral nerves. These EPSPs might thus have been mediated by interneurones intercalated in less specific FRA pathways.

II. *Inhibition of VSCT cells from the cortico-spinal tract*

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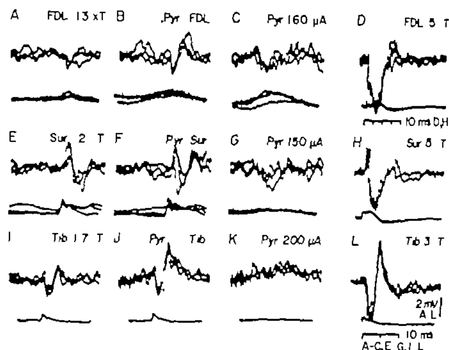


Fig. 6. *Pyramidal facilitation of late EPSP evoked from peripheral nerves. In A D, E H and I L are record* from 3 VSCT cells in which EPSPs preceded by IPSPs were evoked from group I, probably Ib, afferents in FDL (A), from cutaneous afferents in Su (E) and in T b (I) respectively. These were facilitated by t preceding pyramidal stimulation (B, F, J) which by itself evoked either mixed excitatory-inhibitory post synaptic potentials (C, G) or a weak excitation (K). Note that when pyramidal volleys evoked some inhibition *they facilitated not only the EPSPs but also the IPSPs evoked from periphery. The facilitated EPSPs* and IPSPs may be compared with those evoked by stronger stimulation of the same peripheral nerves (D, H, L).

evoked primarily excitation of these cells, the IPSPs were becoming predominant with an increase in intensity and/or number of stimuli, as illustrated in Fig. 3 A, B and G–I and in Fig. 4 A, B. Fairly short latencies (1.5–4.5 ms from the earliest components of the descending volleys) of IPSPs recorded in 9 VSCT cells and their tendency to be shock-locked indicated that they might have been evoked via a disynaptic pathway. Later components of these IPSPs and IPSPs evoked in other cells were, however, clearly polysynaptic. The examples of such late and longlasting IPSPs are in Fig. 3 B and I, Fig. 4 B and H, Fig. 7 C and Fig. 8 L.

2. *Relation between IPSPs of pyramidal and of peripheral origin.* If VSCT cells receive collateral information from last order interneurones of various spinal reflex paths which are under pyramidal control, the IPSPs evoked in these cells by volleys in the cortico-spinal tract may be mediated by inhibitory interneurones interposed in any of these paths. Interneurones more directly influenced by the pyramidal tract should, however, contribute more to inhibition of the VSCT neurones than interneurones excited via longer polysynaptic pathways because with increasing length of interneuronal chains the probability of transmission to last order interneurones would decrease. Accordingly the last order interneurones of di- or trisynaptic inhibitory pathways from Ib or from cutaneous afferents might contribute with stronger and earlier inhibitory effects on VSCT cells since these interneurones are directly or disynaptically excited by cortico-spinal tract fibres (Lundberg, Norrsell and Voorhoeve 1963, Lundberg and Voorhoeve 1962). The collateral effects mediated by inter

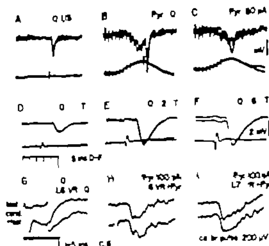


Fig. 7 Relation between IPSPs of pyramidal and of group Ia origin. In A–F and G–I are records from two VSCT cells in which long and short latency IPSPs were evoked by pyramidal stimulation (C and upper traces in H and I) respectively. Test IPSP in A was evoked from group Ia afferents in Q (cf. A and D–F which show that its amplitude was maximal before the strength of Q stimulation became maximal for group I afferents). It was greatly facilitated when combined with pyramidal stimulation (B). Test IPSP evoked in the second VSCT cell from group I afferents (upper trace in G) was depressed by conditioning stimulation in L6 VR (lower trace in G), while the IPSPs of cortical origin evoked in the same cell (upper traces in H, I) were not influenced by stimulation of either L6 or L7+S1 VRs (lower traces in H and I)

neurones of the reciprocal Ia inhibition might be weaker and appear with longer latency in view of a polysynaptic coupling between them and the cortico-spinal tract fibres (Lundberg, Norrsell and Voorhoeve, 1962, Hultborn and Udo 1972). To verify this conclusion the short latency IPSPs of pyramidal origin were tested for depression by conditioning stimulation of ventral roots. If they were mediated by Ia inhibitory interneurones their amplitude should decrease following activation of Renshaw cells, as in other cases of effects mediated by the same interneurones (Hultborn, Jankowska and Lindström 1971 Hultborn and Udo 1972, Gustafsson and Lindström 1973 Jankowska, Lundberg and Stuart 1973 Jankowska and Tanaka 1974, Hultborn, Illert and Santini 1975, Illert and Tanaka 1976). As illustrated in Fig. 7 H and I we failed to find any depression of the short latency IPSPs evoked in 7 VSCT cells from the cortico-spinal tract although IPSPs evoked in them from group Ia afferents were as effectively depressed (Fig. 7 G) as described previously for both α-motoneurones (Hultborn *et al.* 1971) and VSCT cells (Gustafsson and Lindstrom 1973). Cortical facilitation of IPSPs evoked from group Ia afferents in 7 out of 9 tested VSCT cells, was observed only at fairly long conditioning-testing intervals (Fig. 7 A–F). Only late IPSPs of cortical origin could thus be likely to be mediated in VSCT cells by the Ia inhibitory interneurones, the short latency IPSPs being mediated by other interneurones.

Effects of excitation of interneurones interposed in reflex paths from group Ib and from cutaneous afferents by volleys in the pyramidal tract is evidenced by strong facilitation of IPSPs evoked in VSCT cells from these afferents. Facilitation of IPSPs evoked from group Ib afferents is illustrated in Fig. 8. The test IPSP was evoked from Pl nerve (A and D with

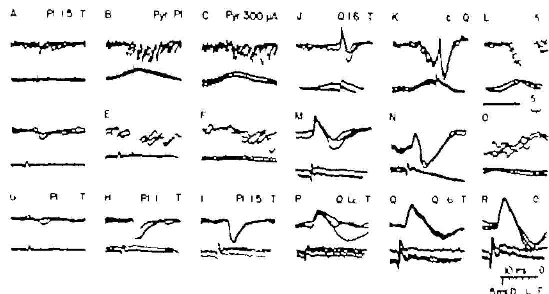


Fig. 8. Facilitation of IPSPs evoked from group Ib afferents by pyramidal volleys. In A–I and J–R are records from two VSCT cells in which disynaptic IPSPs were evoked from group Ib afferents in Pl (cf. records in A, D with those in G–I which show that no IPSP was evoked from lowest threshold group Ia afferents and that it grew until stimulus strength became maximal for group I afferents) or in Q (cf. records in J–M with those in P–R which similarly show that the IPSP was evoked by higher threshold group I afferents). Records in B, E and in K, N show facilitation of the IPSPs by a preceding stimulation of pyramidal tract fibres which evoked a weak inhibition by itself

slow and fast sweep speed respectively) when relatively low but not the lowest (cf. G–J) threshold fibres were stimulated, or from Q nerve (J–M) by stimuli within the group Ib range as indicated by its fairly high threshold and growth over the whole group I range of stimulus strength. When combined with pyramidal (B, E) or with cortical stimulation (K, N) the same stimuli evoked much larger IPSPs, although the latter were superimposed on IPSPs of cortical origin (seen separately in C, F and L, O). Records in Fig. 9 show facilitation of IPSPs evoked from the FRA, which was particularly strong for IPSPs evoked from afferents in SP (F). Since IPSPs of cutaneous origin were usually most effectively facilitated, interneurones interposed in reflex paths from skin afferents might contribute more to the IPSPs evoked in VSCT cells than interneurones activated from other flexor reflex afferents. However, since IPSPs evoked from high threshold skin and muscle afferents in different nerves were similarly facilitated, it indicates that interneurones shared by common FRA pathways might inhibit VSCT cells as well.

Discussion

The results of the present study show that not only inhibition (Magni and Oscarsson 1961) but also excitation is evoked in VSCT cells following stimulation of cortico-spinal tract fibres at a cortical or at a medullary level. Physical spread of current to other than pericruciate cortical areas could be practically excluded in our experiments by use of intracortical stimuli of 10–20 μA and previous experiments have shown that effects of such stimuli in motoneurones are abolished by pyramidal tract lesions (Asanuma and Sakata 1967). Similar

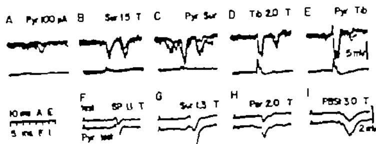


Fig. 9. Facilitation of IPSPs evoked from the FRA by pyramidal volleys. Upper traces in A–E are intracellular records from VSCT cell, lower traces are records from the surface of the spinal cord. In F–I both the upper and the lower traces are intracellular records (averaged). A, weak IPSP following stimulation of pyramidal tract fibres. B, D and upper traces in F–I, IPSPs evoked by stimulation of various hind limb nerves. C, E and lower traces in F–I, facilitation of IPSPs following combined stimulation of the same nerves and of pyramids. Note particularly strong facilitation of IPSP evoked from SP (F).

effects of stimulation of the cortex and of the medullary pyramids (when the pyramidal tract was sectioned a few millimeters rostrally) indicate further that additional effects via other than pyramidal tract cells or fibres, which might have been coexcited at a cortical level or excited via early collaterals of cortico-spinal tract fibres in preparations with intact pyramids, were negligible under our experimental conditions. Any effects relayed by descending systems activated via collaterals of cortico-spinal tract fibres given off below pons level could, on the other hand, be only partly eliminated by contralateral hemisection of the spinal cord and by lesions of the ipsilateral ventral and ventrolateral funiculi. There remains thus a possibility that some of the effects of volleys in the cortico-spinal tract fibres were relayed in lower medulla (see Kuypers 1958) or were evoked via long descending propriospinal systems recently disclosed by Illert, Lundberg and Tanaka (1975) and not only via short propriospinal systems (Lloyd 1941 see also Kostyuk 1971) or interneurones of spinal reflex paths (Lundberg and Voorhoeve 1962). This would not, however in itself speak against VSCT cells receiving collateral information about cortico-spinal control of transmission in spinal reflex paths, if these volleys were similarly relayed in both cases.

Our observations that not only inhibition but also excitation is evoked in VSCT cells by volleys in cortico-spinal tract fibres, and that the same interneurones on the way to VSCT cells are influenced by cortico-spinal volleys and by impulses from various groups of primary afferents are well in keeping with the postulated function of VSCT cells as monitoring transmission through interneurones of spinal reflex paths to motoneurones. We could not, however resolve whether excitation of VSCT cells was collateral to excitation of interneurones which inhibited them or to excitation of other interneurones or motoneurones. Both EPSPs and IPSPs evoked in VSCT cells were usually polysynaptic and to great extent superimposed on each other. It was, therefore, impossible to say if the differences in the latencies of the EPSPs and of the IPSPs corresponded to one or to more synaptic delays. There were either no indications as to whether interneurones mediating inhibition of VSCT cells from cortico-spinal tract fibres were last order interneurones in reflex paths to motoneurones, or else terminated on other interneurones. Negative results of the tests in which conditioning stimulation of ventral roots was tried on the earliest, possibly disynaptic

IPSPs evoked from the pyramidal tract speak against their being mediated by interneurones mediating inhibition of motoneurones from group Ia afferents. Last order interneurones of other reflex paths to motoneurones might have been, however, well responsible for them if group Ib, cutaneous and FRA IPSPs in VSCT cells were collateral to IPSPs evoked in motoneurones, because interneurones which mediated them were strongly influenced by volleys in cortico-spinal fibres.

A particularly effective facilitation of IPSPs evoked from group Ib and from cutaneous afferents indicates that the VSCT might be specially involved in forwarding information on pyramidal control of transmission in reflex paths from these afferents. It might be recalled in this context that in motoneurones cortical facilitation of IPSPs evoked from group Ia afferents and from high threshold muscle afferents is as effective as facilitation of IPSPs evoked from group Ib and from cutaneous afferents (Lundberg and Voorhoeve 1962).

In view of a recent finding that postsynaptic potentials evoked in motoneurones from group Ib and from low threshold cutaneous afferents are at least in part mediated via the same interneurones (Lundberg, Malmgren and Schomburg 1975) a possibility might be considered that information received by VSCT cells about transmission from group Ib and from cutaneous afferents refers in fact to transmission through these common interneurones. If so these interneurones should be strongly excited by volleys in cortico-spinal tract fibres, and a mutual facilitation of IPSPs evoked from group Ib and from cutaneous afferents should occur also in VSCT cells. The VSCT cells might also be informed about transmission through interneurones interposed in more private reflex paths from group Ib or from cutaneous afferents. To these might e.g. belong a subgroup of Ib interneurones whose effects on motoneurones depend on supraspinal facilitation (Hongo, Jankowska and Lundberg 1969).

We wish to thank Dr. Y Padel for participation in preliminary experiment and Mrs. Rauni Larsson for the technical assistance. This work was supported by the Swedish Medical Research Council (Project No 00094) and by Magnus Bergvalls Stiftelse.

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to the hydrostatic pressure exerted by a column of blood of similar height. This gives the possibility to test whether increase in venous or arterial transmural pressures elicits the "vasoconstrictor response" observed in human subcutaneous adipose tissue.

Methods

The experiments were carried out on 6 healthy subjects and 2 patients with venous insufficiency of the legs.

Blood flow in subcutaneous adipose tissue was measured at the lateral malleolus by the local [133]Xenon washout technique (Sejrsen 1971). [133]Xenon dissolved in isotonic saline (0.1 ml) was injected intracutaneously and the measurements were started about 90 min after the injection. At this time the remaining activity was exclusively located in subcutaneous adipose tissue (Sejrsen 1971).

Measurements were undertaken with the subject placed in supine position with the leg placed 1) horizontally $f_{(rest)}$, 2) lowered about 50 cm below the midaxillary line, $f_{(lowered)}$, and finally with the leg placed horizontally $f_{(rest)}$.

Activation of the vein pump was induced by letting the subject tip the foot up and down continuously and the measurements were repeated as described above. In order to test possible change in the vasoconstrictor activity in subcutaneous tissue during exercise the following control expt. was performed. Venous pressure was elevated in the lowered leg during exercise by inflating a cuff placed on the thigh to 40 mmHg.

Venous pressure during the different conditions was measured directly in a superficial vein dorsally on the foot.

In one subject mean arterial pressure was measured directly in an artery dorsally on the foot during the different experimental conditions.

In all subjects arterial pressure was measured on the upper arm by a cuff and mean arterial pressure was calculated as diastolic pressure plus one third of the pulse amplitude.

Calculations and statistics

Blood flow in subcutaneous adipose tissue was calculated as described previously (Henriksen 1976 b). Relative blood flow during lowering, $f_{(lowered)}/\bar{f}_{(rest)}$, was calculated. $f_{(lowered)}$ denotes the perfusion coefficient (ml/100 g min) obtained with the leg lowered and $\bar{f}_{(rest)}$ denotes the average value of the two reference measurements obtained just before and after the test.

From the calculated $f_{(lowered)}/\bar{f}_{(rest)}$ obtained in a single expt. the measured venous pressures and measured or estimated arterial pressures relative vascular resistance, $R_{(lowered)}/\bar{R}_{(rest)}$ was calculated.

Significance tests were performed by means of Student's t-test for paired samples. As level of significance was chosen 0.05.

Results

Normals. An example of the [133]Xenon washout curves obtained in one subject is shown in Fig. 1.

During *resting* conditions lowering of the area under study on the leg to a level 44 cm below mid-axillary line caused an increase in venous pressure of about 34 mmHg in average corresponding to the increase in hydrostatic pressure exerted by a blood column of similar height (Fig. 2).

In this position blood flow in average decreased 52 per cent and total vascular resistance increased in average by 136 per cent (5 expts.) (Fig. 2).

Exercise performed with the leg placed horizontally caused a decrease in venous pressure of 5 mmHg. $f_{(rest)}$ was not changed significantly $p > 0.1$ (Fig. 3). During lowering and exercise re on the foot only increased by 8 mmHg (Fig. 2). Contrary to the resting

Acta physiol. scand. 1977 100 14–21

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Effect of "Vein Pump" Activation upon Venous Pressure and Blood Flow in Human Subcutaneous Tissue

By

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Received 19 July 1976

Abstract

Henriksen, O and P Sejrsen *Effect of vein pump activation upon venous pressure and blood flow in human subcutaneous tissue* Acta physiol scand 1977 *100* 14–21

The effect of "vein pump activation upon superficial venous pressure and blood flow in human subcutaneous d pose tissue was studied in 6 normals and 2 patients with venous insufficiency Blood flow in subcutaneous tissue was measured at the lateral malleolus by the local ^{133}Xenon washout technique, with the subject placed in a supine position. During passive lowering of the leg blood flow decreased 50 per cent and total vascular resistance increased 136 per cent. Activation of the ein pump by continuously tipping the foot up and down caused decrease i enous pressure of 5 mmHg in horizontal position Venous pressure increased only by 8 mmHg when the leg was lowered during exercise In this situation blood flow remained constant corresponding to an increase i vascular resistance of 42 per cent. However increasing venous pressure to 28 mmHg by venous stasis in the lowered leg during exercise caused an dditional increase in vascular resistance of 82 per cent In the patients with venous insufficiency exercise did not prevent the decrease in blood flow during loweri g of the leg. Hence venous pressure elevation of 25 mmHg or more caused an additional ncrease i vascular resistance in subcutaneous tissue "vasoconstricto response It is concluded that this "vasoconstrictor response depends on a asoconstrictor impulse transmission from veins to arterioles, veno-arteriolar reflex.

Blood flow in human subcutaneous adipose tissue decreased about 50 per cent due to an increase in vascular resistance when vascular transmural pressure was elevated 25 mmHg or more by lowering, external negative pressure, or venous stasis (Henriksen Levin Nielsen and Paaske 1973 Paaske and Henriksen 1975 Henriksen 1976 d) Recent studies indicated that the "vasoconstrictor response to increase in vascular transmural pressure is due to a local sympathetic reflex mechanism (Henriksen and Alsner 1975 Henriksen 1976 a, b, c and d) The "effector site" of the reflex is probably located in the arterioles (Landis 1929 Haddy and Scott 1964 Richardson and Zweifach 1970 Baez *et al* 1974)

It is not known whether the "receptor site" of the reflex is located in veins or arteries.

Activation of the "vein pump" might prevent an increase in venous pressure during passive lowering of a leg whereas arterial pressure in this situation increases corresponding

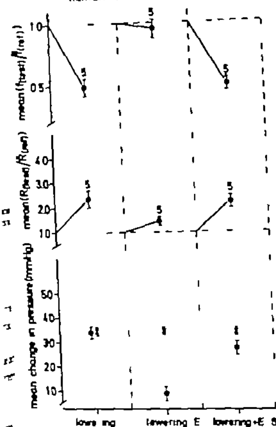


Fig. 2

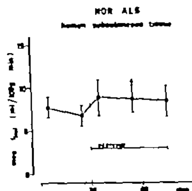


Fig. 3

Fig. Mean relative blood flow $f_{(lower)}/\bar{f}_{(rest)} \pm 1$ S.E., where $f_{(lower)}$ denotes the perfusion coefficient (ml/100 g min) during lowering and $\bar{f}_{(rest)}$ denotes the average value of the two $f_{(rest)}$ obtained just before and after lowering of the leg. Mean $R_{(lower)}/\bar{R}_{(rest)} \pm 1$ S.E. denotes calculated relative vascular resistance during lowering. ● mean change in venous transmural pressure (mmHg) ± 1 S.E. during lowering. X estimated mean change in arterial pressure during lowering. E denotes exercise (dorsal pping of the foot) and S denotes venous stasis of 40 mmHg.

Fig. 3. Mean $f_{(rest)} \pm 1$ S.E. obtained at rest and during exercise in normals.

Exercise did not prevent an increase in venous pressure in the lowered leg. In this position blood flow decreased and vascular resistance increased similar to that observed in the lowered resting leg ($f_{(lower)}/\bar{f}_{(rest)}$ 0.48, $R_{(lower)}/\bar{R}_{(rest)}$ 2.08).

Discussion

The main result of the present study is that exercise did abolish "the vasoconstrictor response" to increases in vascular transmural pressure in the lowered leg in normals. Th

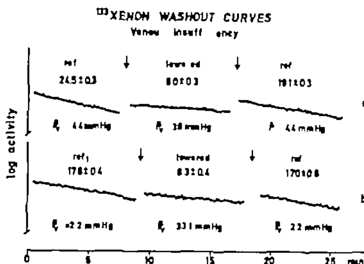


Fig. 1 Example of ^{133}Xenon washout curves obtained in one normal subject, with the leg placed horizontally, ref and ref and with the leg lowered 43 cm below the mid-axillary line. *a.* during resting conditions; *b* during exercise, E, by-continously tipping the foot up and down *c.* during exercise, E, plus venous stasis of 40 mmHg, S, in the lowered position.

The figures above the curves denote the ^{133}Xenon washout rate constants 10³ (min⁻¹)±1 S.D. P denotes mean venous pressure measured directly in a superficial vein i mmHg.

conditions blood flow now remained constant in the lowered leg and total vascular resistance increased in average by 42 per cent corresponding to the increase in arterial perfusion pressure head. When venous pressure in the lowered leg during exercise was increased in average by 28 mmHg by inflating a cuff on the thigh to 40 mmHg blood flow in average decreased 50 per cent. Vascular resistance increased in average by 124 per cent, largely corresponding to the observed increase in vascular resistance in the lowered resting leg.

In one subject a nerve block was induced by infiltrating the skin 5 cm proximal to the area under study with lidocaine and noradrenaline in order to avoid interference of central reflex mechanisms. Skin temperature was kept at 34–35°C by external heating. The result of one experiment is shown in Fig. 4 Lowering the area under study on the resting leg from 20 cm to 30 cm below mid-axillary line caused a decrease in blood flow of 50 per cent, Fig. 4. As arterial and venous pressures measured directly increased in parallel, this corresponded to an increase in vascular resistance of 100 per cent. Exercise increased reference blood flow by 10 per cent. When the exercising leg was lowered 60 cm below mid-axillary line, blood flow increased by 10 per cent. Lowering of the leg caused an increase in directly measured arterial mean pressure corresponding to the increase in hydrostatic pressure exerted by a column of blood of similar height. Exercise did not change arterial mean pressure nor did venous stasis of 40 mmHg. As arterial perfusion pressure head increased about 28 per cent this corresponded to an increase in total vascular resistance of only 20 per cent (autoregulatory response) compared to 100 per cent in the lowered resting leg.

Venous insufficiency The results obtained in one of the two patients are shown in Fig. 5. During resting conditions blood flow decreased by 69 per cent and vascular resistance increased by 228 per cent. Venous pressure now increased corresponding to the increase in hydrostatic pressure exerted by a column of blood of similar height.

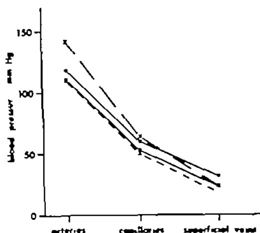


Fig. 6. This diagram shows the blood pressures in the different parts of the vascular bed during the different conditions presented in Fig. 4. Arterial and venous mean pressures were measured directly. Estimation of capillary pressures are based on the results of Levick and Michel (1978). The data presented are considering the situation before compensatory changes in vasoconstrictor activity take place the situation before active changes in the diameter of the vessels have taken place. —·— Pressures in the resting leg lowered 20 cm below the mid-axillary line, lower curve, and 30 cm below the mid-axillary line, upper curve. — — Pressures in the leg during exercise lowered 20 cm below the mid-axillary line, lower curve, and lowered 60 cm below the mid-axillary line, upper curve. For details see text.

fect of exercise might be due to activation of the "vein pump" preventing the increase in mous transmoral pressure during lowering of the leg.

Vascular resistance increased in average by 136 per cent in the lowered resting leg. In this tuation arterial and venous pressures increased in parallel (Fig. 2). During exercise blood low remained constant in the lowered position where venous pressure only increased about mmHg. Vascular resistance increased in average by 42 per cent corresponding to the acrease in arterial perfusion pressure head (autoregulation). Elevation of venous pressure n the lowered leg during exercise induced an additional increase in vascular resistance by l2 per cent in average.

Thus an increase in venous transmural pressure elicited an additional increase in vascular esistance. This indicates that the "vasoconstrictor response (Henriksen 1976 d) depends upon vasoconstrictor impulse that is somehow transmitted from veins to arterioles, superimposed on the autoregulatory response. However exercise might cause a change in metabolism and liberation of metabolites in subcutaneous tissue affecting reactivity of vascular smooth muscle cells. Exercise did not change reference blood flow significantly (Fig. 3). The "vasoconstrictor response was present during exercise when venous transmural pressure was increased by venous stasis in the lowered leg. Similarly in patients with venous insufficiency exercise did not abolish the "vasoconstrictor response"

It is, therefore, concluded that the effect of exercise upon the "vasoconstrictor response" in normals is probably not due to changes in metabolism affecting myogenic activity The effect is more likely due to activation of the "vein pump" which prevents the increase in venous pressure during lowering.

Thus the "vasoconstrictor response seems to depend upon a vasoconstrictor impulse transmission from veins to arterioles.

The abolishment of the "vasoconstrictor response" during exercise might be due to a reduced intrinsic myogenic activity compared to the lowered resting leg. The increase in transmural pressure in the terminal arterioles during lowering the exercising leg might be reduced secondary to the reduced increase in venous pressure.

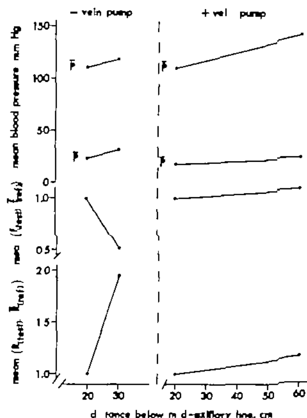


Fig. 4 This diagram shows the effect lowering the leg upon mean arterial pressure $\bar{P}$ mean venous pressure $\bar{P}_v$ relative blood flow in subcutaneous tissue, $f_{(test)}/f_{(ref)}$ and relative total vascular resistance, $\bar{R}_{(test)}$ $\bar{R}_{(ref)}$ obtained in one subject. The effect lowering the resting leg (– vein pump) from 20 cm to 30 cm below the mid-axillary line is shown to the left side and the effect of lowering the leg during exercise (+ vein pump) from 20 cm to 60 cm below the mid-axillary line is shown to the right. Mean arterial and mean venous pressures measured directly on the dorsal region of foot.

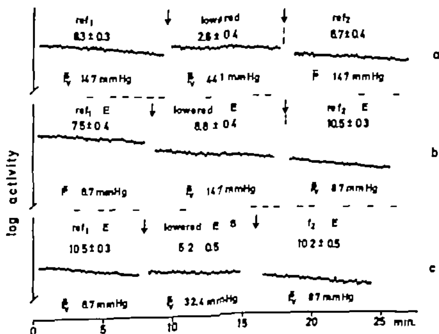


Fig. 5 133Xenon washout curves obtained in one patient with venous insufficiency For symbols see Fig

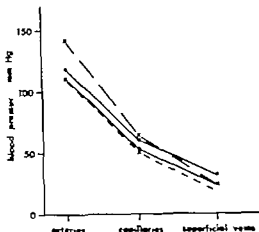


Fig. 6. This diagram shows the blood pressures at the different parts of the vascular bed during the different conditions presented in Fig. 4. Arterial- and venous mean pressures were measured directly. Estimation of capillary pressures are based on the results of Levick and Michel (1979). The data presented are considering the situation before compensatory changes in vasoconstrictor activity take place / the situation before active changes in the diameter of the vessels have taken place. — — Pressures in the resting leg lowered 20 cm below the mid-axillary line, lower curve, and 30 cm below the mid-axillary line, upper curve. — — Pressures in the leg during exercise lowered 20 cm below the mid-axillary line, lower curve, and lowered 40 cm below the mid-axillary line, upper curve. For details see text.

effect of exercise might be due to activation of the "vein pump" preventing the increase in venous transmural pressure during lowering of the leg.

Vascular resistance increased in average by 136 per cent in the lowered resting leg. In this situation arterial and venous pressures increased in parallel (Fig. 2). During exercise blood flow remained constant in the lowered position where venous pressure only increased about 8 mmHg. Vascular resistance increased in average by 42 per cent corresponding to the increase in arterial perfusion pressure head (autoregulation). Elevation of venous pressure in the lowered leg during exercise induced an additional increase in vascular resistance by 82 per cent in average.

Thus an increase in venous transmural pressure elicited an additional increase in vascular resistance. This indicates that the "vasoconstrictor response" (Henriksen 1976 d) depends upon vasoconstrictor impulse that is somehow transmitted from veins to arterioles, superimposed on the autoregulatory response. However exercise might cause a change in metabolism and liberation of metabolites in subcutaneous tissue affecting reactivity of vascular smooth muscle cells. Exercise did not change reference blood flow significantly (Fig. 3). The "vasoconstrictor response" was present during exercise when venous transmural pressure was increased by venous stasis in the lowered leg. Similarly in patients with venous insufficiency exercise did not abolish the "vasoconstrictor response".

It is, therefore, concluded that the effect of exercise upon the "vasoconstrictor response" in normals is probably not due to changes in metabolism affecting myogenic activity. The effect is more likely due to activation of the vein pump which prevents the increase in venous pressure during lowering.

Thus the "vasoconstrictor response" seems to depend upon vasoconstrictor impulse transmission from veins to arterioles.

The abolishment of the "vasoconstrictor response" during exercise might be due to a reduced intrinsic myogenic activity compared to the lowered resting leg. The increase in transmural pressure in the terminal arterioles during lowering the exercising leg might be reduced secondary to the reduced increase in venous pressure.

Site of the transmural pressure changes eliciting the "vasoconstrictor response

In humans placed in a supine position average capillary pressure in the nail bed of the big toe was about 37 mmHg at a skin temperature of 34 C (Levick and Michel 1976).

The directly measured arterial and venous pressures from the expts. presented in Fig. 4 and estimated capillary pressures based on Levick s and Michel s data are shown in Fig. 6. The lower solid line in Fig. 6 denotes the blood pressures in the different parts of the vascular bed with the foot lowered 20 cm below the mid-axillary line, the difference between capillary and venous pressures being 28 mmHg. Now considering the pressure changes in the capillaries before compensatory changes in vasoconstrictor activity take place *i.e* no active change in diameter of the vessels and assuming that changes in vasoconstriction largely occurs in the precapillary part of the vascular bed the changes in capillary pressure during the different experimental conditions presented in Fig. 4 can be deduced

Lowering the resting leg from 20 cm to 30 cm below the mid-axillary line would cause an equivalent increase in transmural pressures in all parts of the vascular bed of about 7 7 mmHg giving a capillary pressure of about 60 mmHg, upper solid line. This increase in vascular transmural pressure exceeded the threshold level (Henriksen 1976 d) and elicited an increase in vascular resistance of 100 per cent (Fig. 4). When the leg was placed 20 cm below the mid-axillary line during exercise venous pressure decreased from 23.5 mmHg to 18 mmHg Blood flow increased by 10 per cent compared to the resting leg and capillary pressure was calculated to be about 50 mmHg, lower broken line. Lowering the exercising leg to 60 cm below the mid-axillary line caused an increase in arterial perfusion pressure head of 28 per cent as venous pressure only increased by 6 mmHg. Before compensatory changes in vascular constrictor activity take place blood flow will increase by 28 per cent and consequently increase the pressure fall through the vascular bed Thus the pressure gradient from capillaries to veins will increase from 32 mmHg to 40 mmHg giving a capillary pressure of about 63.5 mmHg, upper broken line. This indicates that the increase in transmural pressure and consequently the tendency of passive distension of the terminal arterioles was not reduced in the expts. with activation of the 'vein pump which almost prevent the increase in venous transmural pressure. In this situation vascular resistance only increased by 20 per cent. This suggests that the vasoconstrictor impulses underlying the vasoconstrictor response to increase in vascular transmural pressure are not due to changes in transmural pressure in the terminal arterioles, but in venous vessels This is compatible with the previous findings that these vasoconstrictor impulses appear to be transmitted locally in sympathetic adrenergic fibres, forming a local veno-arteriolar reflex (Henriksen and Alsner 1975 Henriksen 1976 a, b, c, and d)

Implications

During muscular exercise the distribution of cardiac output is changed Blood flow in resting muscles, adipose tissue, cutaneous tissue and viscera is reduced. In the exercising legs venous pressure is kept low due to the venous pump activation, indicating that the local veno-arteriolar reflex mechanism is not involved. The mechanism responsible is presumably due to a centrally elicited increase in sympathetic vasoconstrictor activity (Rowell 1974)

In patients with venous insufficiency—contrary to normals—blood flow in subcutaneous tissue of legs is reduced by 50 per cent during walking. This is probably also the case in cutaneous tissue. This might play a part in development of crural ulcers in these patients.

The authors wish to express their gratitude for valuable help to Dr Erik Jacobsen, Dept. of Anaesthesiology Rigshospitalet, Copenhagen, Denmark.

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Intrarenal Distribution of Blood Flow and Glomerular Filtration during Chronic Unilateral Ureteral Obstruction

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Abstract

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Paired hydronephrotic (HN) and hypertrophic (HT) rat kidneys were studied after 6 days with complete unilateral ureteral obstruction without exposing the kidneys. Total HN renal blood flow (RBF), estimated by total microsphere (MS) uptake and from local ^{125}I-antipyrine (Ap) uptake, averaged about 3/4 of control HN kidney GFR was reduced to about 1/2 of control level as estimated from inulin clearance of HT kidney times the HN to HT ratio for mean single nephron filtration rate, determined by ^{14}C-ferrocyanide. Whereas blood flow (Ap) was proportionately reduced in outer and inner cortex (OC and IC), fractional flow to the outer medulla (OM) was doubled as compared to controls ($p < 0.01$). Filtration was well preserved in deep as compared to superficial glomeruli with a smaller deep nonfiltering fraction ($p < 0.02$). Thus the results oppose the current concept that HN is characterized by disproportionate circulatory damage to IC and OM with little or no filtration in deep nephrons. In HT kidneys average RBF (MS) and GFR rose by about 1/2. Whereas total blood flow (Ap) rose proportionately in OC and IC, it remained at control level in OM indicating dissociation between the total RBF and GFR and the effective blood flow to the OM zone.

The nature of renal failure in total chronic unilateral ureteral obstruction (UUO) has been subject to few functional studies This may partly be due to difficulties in applying conventional clearance and micropuncture techniques without urine flow and without detectable tubular flow in a large fraction of the nephrons. Functional damage during UUO in rats, as indicated by prolonged proximal tubular passage time or lack of passage of lissamine green, first appears in the perihilar region and spreads with time towards the suprapapillary central core of the hydronephrotic (HN) kidney (Huguenin *et al* 1974) Nephron function is greatly variable and a significant fraction of glomeruli is without detectable filtration as demonstrated by micropuncture (Yarger *et al* 1972, Wilson 1972, Jaenike 1972, Harris and Yarger 1974) and by ferrocyanide techniques (Harris and Yarger 1974 Wilson 1975 a)

General agreement seems to exist that renal concentrating ability is impaired after released UUO when the contralateral kidney is intact After release of partial UUO with ipsilateral GFR reduced by about 50% or more, filtration is best preserved in superficial nephrons

(Wilson 1972, 1975 a, b, Harris and Yarger 1974). Accordingly the postobstructive loss of concentrating ability might be attributed to disproportionately impaired deep nephron function, which again could be due to medullary circulatory damage. Such damage might involve continued vasa recta flow with disproportionately reduced peritubular capillary flow in the inner cortex and outer medulla as suggested by Harris and Yarger (1974) from qualitative silicone rubber injection and ferrocyanide studies.

The literature on experimental ureteral obstruction is somewhat confusing because of the many different models studied, including obstruction of one or both ureters, partial or complete of varying duration, and with or without intact contralateral kidney. Moreover the majority of reports deals with the postobstructive stage only and may thus well reflect response to release of obstruction rather than functional characteristics of the obstructed kidney.

The aim of the present study was to test the hypothesis that disproportionate functional damage to the deep nephrons and the medulla is characteristical for the UUO rat kidney while avoiding possible effects of obstruction release *per se*. We therefore decided to measure local blood flow in outer and inner cortex and outer medulla and to determine the distribution of filtrate between deep and superficial nephrons in kidneys with chronic, complete UUO and intact contralateral kidney. Since this HN kidney model is without excretory function we used methods that require neither collection of fluid derived from filtrate nor handling of the kidney *in vivo*.

Methods

Female Wistar rats weighing 180–250 g given standard rat food containing 0.6 g sodium chloride per 100 g and tap water were used. Unilateral ureteral ligation (UUL), completely occluding the left ureter, was made close to the bladder through a small suprapubic incision during ether anaesthesia.

Three groups of rats were studied 5– days after *UUL*. The relative filtration rates of superficial and deep glomeruli (S/D SNGFR) were determined in the first UUL group consisting of 6 rats, 3 of which were volume expanded by infusion of isotonic saline. Results on the hypertrophic kidneys of this group has been published previously (Clausen 1974). The ^{14}C ferrocyanide technique for S/D SNGFR measurement described in a previous report (Clausen and Tyssebotn 1973).

The second UUL group, consisting of 6 rats, were used for local renal blood flow measurement by the ^{125}I-antipyrine (Ap) method (Kety 1951) as recently described by Hope, Clausen and Aukland (1976).

The rats in these two groups were anesthetized by intraperitoneal injection of Nembutal, 50 mg/100 g b. A catheter (PE 10) was introduced through a femoral artery with its tip placed in the aorta about 3 cm below the renal arteries. This was used for continuous blood sampling. A second femoral arterial catheter was used for blood pressure recording and a femoral venous catheter for infusion of Ap or ferrocyanide. After tracer infusion the kidneys were excised, snap frozen and samples for local filtration and blood flow measurements were dissected. A minimum of 18 nephrons of each type was dissected from 9 different sectors of radial kidney slices. The ^{14}C radioactivity of distal tubular segments represents the amount of non-filtered ferrocyanide adhering to the tubules. To obtain net radioactivity of the filtered amount, this distal blank was subtracted from proximal tubular radioactivity in individual deep and superficial nephrons.

Tissue samples from outer and inner halves of cortex (OC and IC) and outer medulla (OM_1 and OM_2) were dissected from about 10 sectors in each kidney. Blood flow in ml/min g was calculated for each sample according to the method of Kety (1951) from the curve describing arterial Ap concentration with time and the tissue concentrations in each experiment.

RBF of the two kidneys, as obtained by the Ap method was checked by microsphere (MS) technique in a third UUL group consisting of 8 rats. Under Nembutal anesthesia, a PE 50 catheter with sidehole was placed through the left carotid artery with its tip in the descending part of the aortic arch. About 150 000 MS of 15 ± 5 μ diameter labelled with ^{85}Kr (NEN) was injected as a bolus through the carotid catheter. A second catheter (PE 10) with its tip in the abdominal aorta was used for blood sampling at a constant rate

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of 20 µl/s by a syringe pump, beginning 5 s before MS injection and lasting for 30 s. Blood pressure was continuously recorded from a third arterial catheter. The RBF of the two kidneys were calculated from the radioactivity of MS taken up by the kidneys (in the order of 10^4 cpm) and the radioactivity of sample blood.

In order to test the possibility that hypertrophia occurring during UUL differed from that following nephrectomy a fourth experimental group consisting of 4 rats was investigated by the Ap method 6 days after unilateral nephrectomy.

Several control groups consisting of rats with both kidneys intact were needed in order to evaluate functional changes following UUL and nephrectomy. For S/D SNGFR and GFR we used previous results (Clausen and Tyssebotn 1973) supplemented by those obtained in 5 rats studied by the ferrocyanide method at the time of the UUL experiments.

For blood flow (Ap) and its distribution results from a recent study by Hope, Clausen and Aukland (197?) provided the control data. RBF was also determined by MS in both kidneys of 5 control rats in the present study.

Renal wet and dry weights were determined in 12 pairs of HN and HT kidneys and in 12 control kidneys according to usual standard methods. Special care was taken to remove pelvic fluid from the HN kidney by cutting it in halves and blotting on filter paper before weighing.

Results

Rats with UUL or unilateral nephrectomy had an average AP of 135 (SD 11 mm Hg, n = 27) whereas $\overline{AP}$ of the control groups (both kidneys intact) averaged 122 ± 11 mm Hg (p < 0.00?, n = 30). In some expts. a transient fall in AP was observed during tracer infusion. These expts. are not included in the present report.

Hypertrophic kidneys

The average increase of renal wet weight due to hypertrophy was 30% with unaltered dry matter/water content ratio as compared to controls (Table I). Whereas average effective local blood flow with respect to Ap increased insignificantly in the cortex, it decreased by 30% ($p < 0.05$) in the outer medulla (Table II). Total RBF was calculated from the local flow data assuming that zonal weights were 42% (OC), 28% (IC) and 20% (OM) of total kidney weight. The average increase of total RBF was 36% during hypertrophia. The finding of a significantly increased RBF was confirmed by the microsphere method showing an average increase of 47% as compared to controls (Fig. 3, Table IV).

Hydronephrotic kidneys

Hydronephrosis developed in the left kidney in all rats within 5–7 days after UUL. Marked dilatation of the pelvis was associated with distension of cortex and medulla, particularly

TABLE I. Renal weight, water and dry matter content ± S.D. in hydronephrotic (HN) and hypertrophic (HT) kidneys as compared to controls (C).

	Kidney wet weight		Dry weight in % of wet weight	ΔDry matter per kidney	ΔH_2O per kidney
	g/100 g BW	% of control			
C	0.3170 ± 0.033	100	22.4 ± 0.71	0	0
HT	0.4130 ± 0.029	130	22.8 ± 0.86	+ 32	29
HN	0.6619 ± 0.091	209	15.1 ± 1.60	41	+ 128

TABLE II. Local blood flow in ml/min g ± S.D. in control (C) kidneys, hypertrophic (HT) kidneys in rat with unilateral ureteral ligation (UUL) or nephrectomy (UNE) and in the paired hydronephrotic (HN) kidneys of the UUL rats

		OC	IC	OM_1	OM_2	OM	IC/OC	OM/OC
C	13	7.09±1.13	4.65±0.83	2.17±0.37	0.89±0.25	1.66±0.32	0.66	0 4
HT/UUL	6	7.64±1.15	4.97±0.90	1.71±0.40	0.60±0.18	1.27±0.31	0.65	0.17
HT/UNE	4	7.62±0.75	4.60±1.21	1.76±0.45	0.67±0.20	1.32±0.38	0.60	0.17
HN/UUL	6	2.43±0.66	1.77±0.52	—	—	1.06±0.54	0.7[illegible]	0.43

OC and IC, outer and inner cortex, OM_1 and OM_2 outer and inner halves of outer medulla, OM total outer medulla. *p < 0.05

in the perihilar region, whereas the papilla appeared elongated and narrowed. The ureter was distended down to the ligature and, like the pelvis, filled with a clear fluid.

During UUL, renal weight increased by 109% in the HN kidneys (Table I). Whereas the water content increased in proportion to wet weight in HT kidneys a disproportionately high amount of water corresponding to 228% of control kidney water content, was found in HN kidneys.

Blood flow

Flow distribution among the zones is presented in Table II whereas individual results are illustrated in Fig. 1. The broken lines refer to average zonal flow ratios in controls. The most marked alteration in inter zonal flow distribution in the HN kidneys was a relative increase of OM flow. The outer medulla was not separated in two zones in the HN kidneys since this could hardly be done with reasonable accuracy due to decreased zonal thickness. Thus, the OM zone of HN kidneys corresponds to OM_1 and OM_2 of the HT and control kidneys (*cf.* Table II).

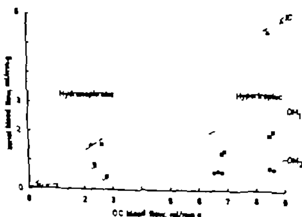


Fig. 1. Zonal blood flow in hydronephrotic and hypertrophic kidneys. Flow in inner cortex (IC) and outer medulla (OM_1 and OM_2, outer and inner halves) as related to outer cortical (OC) flow. IC/OC (triangles), OM_1/OC (squares) and OM_2/OC (circles). Broken lines indicate average ratios in 13 pairs of control kidneys (Hepp et al. 1979). Hypertrophic kidneys in contralateral nephrectomy: open symbols.

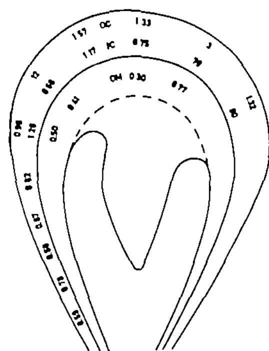


Fig. 2

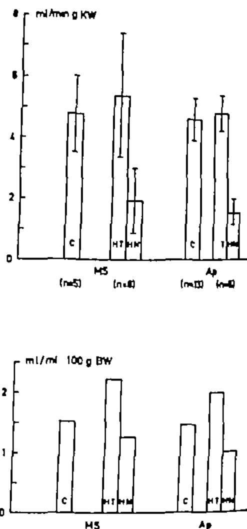


Fig. 3

Fig. 2. Schematic demonstration of local zonal blood flow (ml/min g) in outer and inner cortex (OC and IC) and outer medulla (OM) in hydronephrotic kidney. Note that cortical flow (OC IC) only determined in the most distended part of the kidney ("perihilar region").

Fig. 3. Single kidney blood flow hydronephrotic (HN), hypertrophic (HT) and control (C) kidneys determined by microsphere (MS) and antipyrine (Ap) techniques. Brackets indicate ± S.D.

Local cortical flow declined towards the pedicle in the perihilar region (Fig. 2) averaging *approximately half the suprapapillary level.*

Total RBF of the HN kidneys was calculated from the local flow data assuming that the suprapapillary region constituted 3/4 of renal mass and that the weight increase was uniformly distributed among the zones. Although flow averaged only 1.53 ml/min g HN kidney as compared to 4.62 ml/min g in controls, HN kidney RBF was maintained at 69% of control level (Fig. 3) due to increased kidney weight.

In order to test the validity of the surprisingly high calculated RBF of the HN kidney the RBF of HN and HT kidneys was determined by radioactive MS technique in 8 UUL rats. According to the Ap-results the expected average MS uptake should be 51 in the HN

TABLE III. Total GFR of paired hydronephrotic (HN) and hypertrophic (HT) kidneys and the distribution of filtrate between superficial (S) and deep (D) nephrons.

	S/D SNGFR		GFR					
	HN	HT	ml/min 100 g BW			HN	% of control	
			HN*	HT	Total	% of HT	HN	HT
E	49	80	0.29	0.73	1.02	40	66	166
E	55	77	0.22	0.64	0.88	34	50	145
E	89	87	0.27	0.78	1.05	34	61	177
H	56	130	0.12	0.96	1.08	12	27	218
H	87	95	0.11	0.85	0.96	13	25	193
H	93	80	0.43	0.74	1.17	58	98	168
Mean	72	85	0.24	0.78	1.03	31	55	178
S.D.	20	26	0.12	0.11	0.10	17	27	25

* Assuming no filtration in the perihilar region (see text). E, volume expanded by i.v. infusion of isotonic saline (Clausen 1974). H, hydropenic.

kidney as compared to that of the HT kidney. The average ratio observed was 57%. In 5 control rats the flow difference between paired kidneys averaged less than 10% without correction for paired kidney weight differences. Thus, both the Ap and MS flow measurements indicated that the HN kidney maintained total RBF averaging about 3/4 of control single kidney RBF (Fig. 3).

Glomerular filtration rate

The S/D SNGFR ratio averaged 0.72 in the HN kidneys as compared to 0.85 in the corresponding HT kidneys (Table III) and 0.90 ± 26 (n = 11) in controls. The differences between these ratios are statistically not significant ($p > 0.05$). However, the fraction of single kidney total GFR produced by deep nephrons was higher in HN *than in paired HT* kidneys in 4 out of 6 rats.

The distribution of single nephron ferrocyanide in a pair of UUL rat kidneys is demonstrated in Fig. 4. In contrast *to control and HT kidneys* where no or occasionally a negligible overlap between distal and proximal tubular radioactivity occurs, all HN kidneys had such overlap, on average in 33 (16–60)% and 49 (36–77)% of deep and superficial nephrons respectively.

Since distal tubular radioactivity is derived from nonfiltered 14 C ferrocyanide, overlap would indicate zero filtration (*cf.* Methods). A more conservative estimate of the fraction of nonfiltering glomeruli is obtained from overlap between proximal and *average* distal blank radioactivity indicating stopped filtration in 7 (2–10)% and 11 (4–26)% of deep and superficial nephrons respectively.

Although neither tubular fluid nor urine was sampled from the HN kidney, its total GFR may be estimated from average net proximal tubular radioactivity (filtered ^{14}C ferrocyanide) and the ratio of the corresponding activity in the *contralateral* kidney to its C_{In} (Table II). Assuming equal total numbers of glomeruli in both kidneys and that superficial glomeruli constitute 70%, HN kidney GFR averaged 42% of HT kidney GFR corresponding

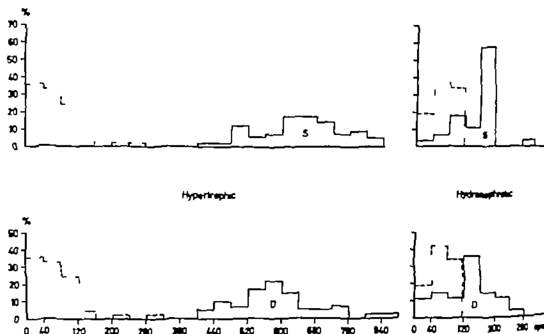


Fig. 4 Distribution of ¹⁴C ferrocyanide radioactivity among superficial (S) and deep (D) proximal tubules and distal tubular segments (broken lines) in a pair of kidneys after 6 days with unilateral ureteral ligation. Abscissa counts per minute (cpm). Ordinate fraction of total number (40 and 28 tubules of each type) in the hypertrophic and hydronephrotic kidney respectively. Overlap between proximal and distal cpm indicate stopped filtration in the hydronephrotic kidney (cf text).

to 75% of control GFR. This is, however possibly an overestimate since the perihilar region had relatively low blood flow and therefore might have a similarly reduced filtration or perhaps no glomerular filtration at all. These possibilities give an average HN kidney total GFR of 62% and 55% of control respectively (Table III). As seen in Table III volume expansion has no detectable effect on HN kidney function

Discussion

Hypertrophic kidneys

The HT kidney of hydropenic rats with 6 days of UUL increased average GFR to 166 of control with a S/D SNGFR of 0 75 (Clausen 1974) as compared to 0 90 in the present and previously studied controls (Clausen and Tyssebotn 1973). Deep nephrons thus increased their SNGFR more than superficial ones. Accordingly one might expect similar changes of cortical blood flow. Average RBF (MS) rose to 147% of control which is not statistically different from the GFR increase. However the distribution of blood flow between the cortical zones was unaltered during HT as compared to controls (Fig. 1). This observation would seem to contradict the previous finding (Clausen 1974) that deep nephrons contribute relatively more to the GFR increase occurring during HT than do superficial ones. Increased filtration fraction in deep glomeruli might explain, at least in part, the apparent discrepancy

Local effective flow declined by 30% in the two outer medullary zones during HT (Table II) but total zonal flow was probably maintained due to the 30% renal weight increase

Table I) which has been reported to be homogeneous throughout the kidney (Wiltrakis 972, McNay and Miyazaki 1973 Kaufman *et al* 1975). Thus, there seems to be a marked lissociation between the total RBF and GFR and the outer medullary blood flow in the present HT kidney model.

The juxtamedullary (JM) nephrons, constituting about 1/3 of the deep nephrons, supply the vasa recta to the medulla as a whole and peritubular capillaries to the outer portion of the outer medulla. Unaltered effective flow to the outer medulla might therefore suggest that these nephrons did not increase their glomerular blood flow However little is at present known as to the quantitative relation between JM glomerular flow and effective outer medullary flow Furthermore, it is likely that the deep cortex receives a portion of its effective flow through capillaries originating from more superficial glomeruli, as recently icussed by Hope et al. (1976).

Thus it cannot be excluded that deep cortical glomerular blood flow and filtration might crease more than total RBF and GFR without increased effective (Ap) flow in deep rtex and outer medulla.

Increased activity of the renin-angiotensin system occurs during UUL in rats as indicated y increased juxtaglomerular granulation index, particularly in the HN kidney (Schurbert *et al.* 975) Thus the presence of an obstructed HN kidney might affect the function of the contralateral HT kidney However this does not seem to be the case with respect to total blood ow and its intrarenal distribution since closely similar results were obtained with UUL nd unilateral nephrectomy (Fig I). Increased renin release might, however explain the 5 mmHg higher AP in the present experimental groups as compared to controls.

Hydronephrotic kidney

The gross anatomy of the present HN kidneys corresponds well to that described by Romanmura (1966) in the identical rat kidney model. The increased kidney weight is compatible with that obtained in UUL rats by Dicker and Shirley (1972) and in other species Lyrdal and Olin 1975) Whereas both wet and dry weight increased by about 30% in the HT kidney the water content of the HN kidney was more than doubled with a corresponding dry weight increase of 41%, *i.e.* significantly more than might be attributed to solutes of renal fluids Thus, the weight increase suggests growth in both kidneys.

Renal blood flow No detectable change in outer to inner cortical flow ratio and increased fractional outer medullary flow (Table II) strongly oppose the working hypothesis that hydronephrosis produced by UUL is characterized by disproportionately impaired deep cortical and outer medullary circulation. The local blood flow of the outer medulla implies such a high rate of Ap-uptake as could not possibly be derived directly from vasa recta bundles alone due to the large distances between the vascular bundles over which Ap would have to diffuse. The present results, therefore, do not seem compatible with the idea forwarded by Harris and Yarger (1974) on continued vasa recta flow with disproportionately reduced peritubular capillary flow n the deeper cortex and outer medulla.

Decreasing local blood flow towards the pedicle (Fig. 2) corroborates the results of Hugosson *et al* (1974) showing lack of passage or disproportionately prolonged passage time for lissamine green in this region of the chronically obstructed rat kidney

TABLE IV Hydronephrotic (HN) and hypertrophic (HT) kidney functions expressed in per cent of contr (C) in hydropenic rats.

	Total				Local		
	Kidney weight	GFR	RBF		D/S SNGFR	Blood flow[b]	
			Ap	MS		IC/OC	OM/OC
C	100	100	100	100	100	100	100
HT	130	166	136	147	120	99	70
HN	209	55[c]	69	84	125[c]	110	200

Deep (D) SNGFR expressed as fraction of superficial (S) SNGFR
[b] Blood flow in inner cortex (IC) and outer medulla (OM) expressed as fraction of outer cortical (OC) flo
Obtained i hydropenic and volume expanded rats (Table III).

Glomerular filtration

The ferrocyanide results demonstrate that the fraction of total GFR derived from dee nephrons was higher in HN than in HT kidneys in 4 out of 6 rats. Together with the findin of less nonfiltration among deep glomeruli as compared to superficial ones in all HN kidney this contradicts the working hypothesis, but agrees well with the observation of maintaine and increased fractional blood flow to the IC and OM zones respectively

To what extent nephrons have ceased to produce filtrate cannot be exactly determined b the present ferrocyanide technique (Clausen and Tyssebotn 1974) However the result leave little doubt that a larger than normal fraction of both deep and superficial proxima tubules contained less than average tubular blank radioactivity (Fig. 4) So far as this ma give a quantitative indication on the extent of nonfiltration it suggests that at least abou 10% of the nephrons in the suprapapillary region had ceased to function.

Both total GFR and effective RBF were surprisingly high considering that the HN kidne was without excretory function for 6 days, and also during experiment Both these function were determined indirectly *i.e* by their HN to HT ratios and by assuming equal fractiona kidney zonal volumes in UUL rats and controls respectively One might therefore suspec that errors are involved in calculation of HN kidney total functions However such error are excluded by satisfactory agreement between Ap and MS results.

We therefore interprete the present results, summarized in Table IV to show that in th HN kidney 1) Total RBF and GFR were reduced by about 1/4 and 1/2 respectively 2) Outer and inner cortical blood flow were proportionately reduced 3) The reduction of SNGFR tended to be less in deep than in superficial glomeruli with less non-filtratio among the deep ones. 4) Outer medullary blood flow was better preserved than cortical flow

Thus, the working hypothesis on disproportionately reduced deep SNGFR and blood flow to inner cortex and outer medulla being characteristical for obstructed kidneys does not apply to the present HN kidney model.

Comparison with previous studies With the exception of Wilson (1975 a) previous authors have generally based their conclusions on deep nephron filtration as calculated from total GFR (C_{In}) and average superficial SNGFR determined by micropuncture. This indirect approach to deep nephron function is by no means safe, since a) superf funct

is heterogeneous with many nephrons unsuitable for micropuncture (not only nonfiltering ones), b) the clearance of inulin probably underestimates GFR in obstructed kidneys due to tubular back-leakage of inulin (McDougal and Wright 1972), c) the disproportionately reduced perihilar function, occurring at least in chronic UUL kidneys, is hardly detected by micropuncture. Each of these conditions will invariably lead to the faulty conclusion that deep nephrons function rather poorly or not at all.

However Wilson (1975 a) reported disproportionately reduced deep nephron function in solitary HN kidneys after relief of partial chronic ureteral obstruction using ferrocyanide technique. It should be emphasized that a S/D SNGFR ratio of 0.95 (Wilson 1975 a) does not *per se* reflect nonfunction or even severely depressed function in deep nephrons relative to superficial ones. Although the average S/D SNGFR ratios are quite similar in HT kidneys in the studies of Wilson (1975 a) and Clausen (1974), 0.73 and 0.75 respectively they are not so for HN kidneys. Wilson, 0.95 present study 0.72. These results should be comparable since total GFR averaged about 0.23 ml/min 100 g BW in both HN kidney models. The discrepancy on filtrate distribution may be related to several differences between the models, possibly to redistribution of blood flow and filtrate in association with natriuresis, following release of solitary kidney obstruction. Release of UUL is known to increase RBF and superficial SNGFR (Murphy and Scott 1966). Wilson (1975 b) confirmed this in a micropuncture study observing 112% increased GFR in solitary HN kidneys. However superficial SNGFR increased simultaneously by a mere 21% suggesting either that punctured nephrons represented only a minor fraction of the outer cortical nephron population or that inner cortical nephrons (30% of the population) were responsible for 85% of the GFR increase. The latter possibility seems to imply however, that deep nephrons did not produce filtrate at all during obstruction. The present results suggest that this is rather unlikely

The most likely explanation to this discrepancy would be that whereas micropuncture gives a valid SNGFR estimate, C_{in} underestimates GFR during obstruction, but to a lesser degree or not at all after its release. This implies a distal tubular location of inulin leakage in the obstructed kidney as previously suggested for a different obstructive kidney model in the dog (Clausen *et al.* 1975). A distal location of tubular leakage may be related to the permanent tubular distention occurring only in the distal portion of the nephrons and in collecting ducts, as observed by Schubert *et al.* (1975) in rats with UUL.

Intracortical microsphere flow distribution has been investigated during postobstructive diuresis after 24 h bilateral ureteral ligation (BUL) in rats by Jaenike (1972) and in dogs by Yarger and Griffith (1974). Common to these studies is unaltered or increased fractional flow to the inner cortex. Feldman *et al* (1974) found increased prerelease inner cortical fractional flow with return to normal after release of 24 h BUL in rats. The present results on chronic UUL apparently agree with these previous studies with regard to well preserved fractional IC blood flow

Thus, we interpret both the direct determinations of intracortical microsphere distribution in obstructed but not HN kidneys (Jaenike 1972, Yarger and Griffith 1974 Feldman 1974) and the present results on HN kidneys to indicate well maintained fractional inner cortical and outer medullary circulation, and deep glomerular filtration, in kidneys with ureteral obstruction.

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[c] Obtained in hydropenic and volume expanded rats (Table III).

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Acta physiol. scand. 1977 100 33–44

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Regulation of Salivary Kallikrein Secretion in the Rat Submandibular Gland

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Received 29 September 1976

Abstract

…AVIK, T. B. and K. M. GAUTVIK. *Regulation of salivary kallikrein secretion in the rat submandibular gland.* Acta physiol. scand. 1977 *100*, 33–44

…indeted parts of rat submandibular glands were compared with regard to their wet weight, total … content and kallikrein activity quantitated by Bz-Arg-OEt-esterase and kallikrein antigenic activity … glands from the same animal were found to be comparable, whereas differences from one animal to … were considerable. One of two paired glands was extirpated and used as control, and the other was …quently subjected to stimulation. Salivary secretion was induced parasympathomimetically (intra…nous injections of pilocarpine, perfusion with acetylcholine and electrical stimulation of the ductal plexus near the gland hilus) or sympathomimetically (cervical sympathetic nerve stimulation with or without administration of or β-adrenergic blocker, perfusion with epinephrine, norepinephrine or iso…renol). The effect was studied by measuring the change in total gland kallikrein content and by quan…on of kallikrein in saliva. A small secretion of kallikrein was always observed. However α-adrenergic …lation was 40 and 1 500 fold more effective in releasing kallikrein than β-adrenergic and parasympa…metic stimulation, respectively. Also, significantly more kallikrein was released by β-adrenergic than …sympathomimetic stimulation. Immunohistochemistry confirmed the observed depletion of kallikrein …wing α-adrenergic stimulation. No alteration in kallikrein localization was observed in stimulated …s.

…vary glands, like most exocrine glands, are known to contain the plasma-kinin forming …yme kallikrein (E.C.3.4.4.21) (Frey *et al.* 1968). Regulation of enzyme synthesis as well as …physiological functions are not fully understood. Studies in the cat submandibular gland …e shown that adrenergic nerve stimulation results in a degranulation of secretory granules … the striated duct cells leading to an almost total depletion of gland kallikrein, suggesting …uctal localization of the enzyme (Garrett and Kidd 1975, Bellenson *et al.* 1968, Barton … *al.* 1975). Kallikrein secretion is in the cat, mediated via stimulation of α-adrenergic …ceptors (Gautvik *et al.* 1974). In this animal, the enzyme has been shown to play a role in … functional hyperemia of the gland. In the rat, less information is available regarding the …pe of cellular receptors responsible for the secretion of kallikrein, even though the rat …bmandibular gland contains more than twenty times as much kallikrein per mg dry weight

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Bz-Arg-OEt-esterase and kinin-forming activity by the sheep anti-rat urinary kallikrein antiserum. T
...ady kinin formation ... submandibular gland homogenate containing 1.0 mg wet gland tissue per ml PBS was produced. Rat kininogen ... as purified according to the method described by Jacobsen (1966). The effect of the gland homogenate to release bradykinin was determined using ... quantitative rat uterus bioassay (Ørstavik 1969). *Kallikrein in the gland homogenate* was precipitated by incubation overnight (4°C) with the sheep antiserum rendered monospecific for kallikrein by absorption ... with cross-reacting submandibular gland antigens (Brandtzaeg *et al.* 1976). Normal sheep serum ... as employed for controls. After centrifugation (30 000 *g* 30 min) the Bz-Arg-OEt-esterase and kinin-forming activity of the supernatant was measured. Protein and esterase activity of the precipitate was estimated by the difference between total amounts prior to precipitation and the remaining supernatant activities after antibody-precipitation.

Protein measurements. All protein measurements ... are performed after the method of Lowry (1951). Bovine serum albumin was used as standard.

Statistical analysis of the results. Analysis of the effects of different treatment upon kallikrein secretion was evaluated by employing Wilcoxon ... test for non-parametric data (... an Elteren 1960). The results presented in tables and figures are given as mean values ± the standard deviation.

Immunohistochemistry. Small pieces of the tissue were fixed *in vitro* in cold 96% ethanol. However, glands stimulated by perfusion were fixed *in vivo* by infusing 96% ethanol (3 ml/min for 3 min, 22°C) into the common carotid artery while cutting the jugular vein to prevent an increase in intraglandular pressure. These glands were post-fixed in ethanol and then followed the usual schedule for tissue processing (Ørstavik *et al.* 1975). *In vivo* fixation was impossible to perform on the control gland from the same animal, drug-perfused glands were therefore compared with buffer-perfused and *in vivo*-fixed control glands from other ... as well as ... with the *in vitro*-fixed control pair gland.

Tissue sections (6 μm) were treated as described previously (Ørstavik *et al.* 1975). Kallikrein distribution ... studied by ... direct immunofluorescence technique employing ... rhodamine conjugate of the immunoglobulin fraction of the above described anti-salivary kallikrein antiserum. The conjugate was purified by ...filtration and ion-exchange chromatography (Brandtzaeg 1973). The selected fraction had an optical density (OD) ratio ($OD_{515\ nm}/OD_{280\ nm}$) of 2.4:1 ... was used at ... concentration of 0.08 mg/ml and contained 1 precipitating unit per ml when tested against ... standardized rat submandibular gland homogenate containing 0.19 mg/ml kallikrein.

Drugs. Pentobarbital (Nembutal sodium ... Abbott Laboratories, London); pilocarpine and epinephrine (NAF Oslo, Norway); acetylcholine chloride (S. A. F. Hoffman-La Roche & Co. A. G.); norepinephrine (...noradrenalin ... Norsk Astra AS, Oslo, Norway); isoproterenol (Isoprenaline sulphas ... Norwegian medical depot); phentolamine (Regitine ... Ciba); propranolol chloride (Inderal® or Propranolol Hydrochloride ... Imperial Chemical Industries Limited). Synthetic bradykinin was ... gift from Sandoz, Switzerland.

Results

Bz-Arg-OEt-esterase activity as ... parameter for glandular kallikrein. The monospecific sheep antiserum gave a normal precipitation curve for kallikrein (Fig. 1 A). At a dilution of 1:3 the maximal amount of protein was precipitated, and the Bz-Arg-OEt-esterase activity in the supernatant of the incubation mixture was completely neutralized (Fig. 1 A). When the antiserum concentration was kept constant (1:3) and the protein concentration of the added gland homogenate varied, a complete inhibition of both esterase and kinin-forming activity was observed when the antigen concentration was less than 0.7 mg wet gland weight per ml (Fig. 1 B). Below this concentration of gland homogenate there was also a linear and parallel relationship between the increase in precipitated protein and precipitated esterase activity (Fig. 1 C). However, above this concentration some esterase activity reappeared, whereas kinin-forming activity was still completely neutralized (Fig. 1 B). This may be explained if the gland homogenate contained other esterases capable of hydrolyzing Bz-Arg-OEt than kallikrein or by the presence of ... glandular inhibitor active against the kinin-forming activity but not against the esterase activity.

as does the equivalent cat organ. Recent immunohistochemical studies localized the enzyme to the ductal elements of the gland and in particular to the secretory granules of the granular tubular cells (Ørstavik *et al* 1975 Brandtzaeg *et al* 1976) The high concentration of kallikrein in the rat submandibular gland suggests an important functional role for this enzyme

The purpose of this investigation was to study the regulation of kallikrein-secretion in the rat submandibular gland employing enzyme measurements in saliva and gland homogenates as well as immunohistochemical examination of intracellular kallikrein.

Materials and methods

Animals. Female and male Sprague-Dawley rats (250–400 g, 10–16 weeks) were used The animals were anesthetized with i.p. injections of pentobarbital (40 mg/kg b. w.t.). All animals were tracheostomized and had spontaneous respiration.

Stimulation of the submandibular gland The submandibular gland of the left side was removed just before the start of stimulation. The cannulated gland of the right side was subjected to stimulation in one of the following ways. *I Close arterial infusion through the common carotid artery by manual injections of a)* acetylcholine (100 μg/ml 30 ml during 5 min), b) epinephrine (100 μg/ml, 30 ml during 5 min), c) norepinephrine (100 μg/ml, 3 ml during 10 min) or d) isoproterenol (100 μg/ml, 15 ml during 20 min). The drugs were dissolved in phosphate buffered saline (PBS 0.01 M Na-phosphate buffer pH 7 4 0.15 M NaCl. Control animals were perfused with PBS only *II* Intraperitoneal injections of pilocarpine (0.4 ml, 4 μg ml. The dose was repeated after 15 min. *III* Electrical stimulation of the ductal nerve plexus near the gland hilus through silver wire electrodes connected to a Grass SD 9 stimulator The gland was stimulated for 30 min at 7.5 V 2 ms duration and 9 5 Hz. *IV* Electrical stimulation of the right cervical sympathetic nerve with 7.5 V 2 ms duration and 9 5 Hz for 20 min. The animals of this group were either given no additional treatment or drugs giving adrenergic α- or β-blockade infused through the jugular vein of the control side prior to stimulation The adrenergic α-blocker (phentolamine, 1 mg/kg b. wt.) was infused 20 min before stimulation of the sympathetic nerve. The β-blocker (propranolol-chloride) was administered in two doses, first injection of 2 mg/kg b. wt. and a second of 10 mg/kg b. wt. 20 min later followed by sympathetic nerve stimulation after an additional 20 min. All infusion fluids were 22°C.

After stimulation, the stimulated like the control gland was processed for immunohistochemistry or frozen at −20°C *for subsequent homogenization and enzyme quantitations.*

Collection of saliva. The main excretory duct of the right submandibular gland was dissected free by removing the digastric and mylohyoid muscles. A polyethylene tubing (PE 10) was inserted into the duct. Saliva was collected into preweighed tubes during the stimulation period and frozen at −20 C for subsequent quantitation of kallikrein antigenic and Bz-Arg-OEt-esterase activity as described below

Quantitation of kallikrein in gland extracts and saliva Tissue homogenization and kallikrein quantitations were done as described previously (Ørstavik *et al.* 1977). The sublingual gland was removed prior to homogenization. Kallikrein was measured by its ability to hydrolyze α N-benzoyl-L-arginine ethyl ester (Bz-Arg-OEt) (Sigma). Kallikrein in saliva was also quantitated immunochemically by single radial immunodiffusion (Brandtzaeg *et al* 1970) A rabbit antiserum raised against purified submandibular gland kallikrein (fraction 4C$_{-4}$ see Fig 1 d and Table I i Brandtzaeg *et al.* 1976) was used at 3 μl/ml gel, and the wells were filled with 10 μl of saliva. Dilutions of an immunochemically standardized submandibular gland homogenate served as internal standards The antigenic activity of gland homogenates was only tested for 7 unstimulated control rats to evaluate differences between the two paired glands. Here a sheep anti-rat urinary kallikrein antiserum (Nustad and Pierce 1974) was used at 10 μl/ml gel, and 5 μl antigen solution was applied per well. Each sample was tested at 3 different dilutions and the mean kallikrein concentration was calculated. In control expts. the cross reacting antigens, esterases A and B (Brandtzaeg *et al* 1976) were added to the internal kallikrein standards.

Immunoelectrophoresis Electrophoresis of 10 μl of salivary samples was followed by diffusion against the above described *anti-salivary kallikrein antiserum for 24 h (Laurell 1966). The plates were washed, dried* and stained with amidoblack

Evaluation of Bz Arg-OEt-esterase activity as kallikrein parameter The usefulness of the Bz Arg-OEt-esterase method as parameter for kallikrein quantitations was evaluated by comparing the Bz Arg-OEt-esterase activity to kallikrein antigenic activity as described above as well as studying the inhibition of

[illegible] Bz-Arg-OEt-esterase and kinin-forming activity by the sheep anti-rat urinary kallikrein antiserum. T[illegible] study kinin formation [illegible] submandibular gland homogenate containing 1.0 mg [illegible] gland tissue per ml PBS was produced. Rat kininogen was purified according to the method described by Jacobsen (1966). The effect of the gland homogenate to release bradykinin [illegible] as determined using [illegible] quantitative rat uterus bioassay (Gautvik 1969). Kallikrein in the gland homogenate was precipitated by incubation overnight (4°C) with the sheep antiserum rendered monospecific for kallikrein by absorption [illegible] with cross-reacting submandibular gland antigens (Brandtzaeg *et al.* 1976). Normal sheep serum was employed for controls. After centrifugation (30 000 g 30 min) the Bz-Arg-OEt-esterase and kinin-forming activity of the supernatant [illegible] as measured. Protein and esterase activity of the precipitate [illegible] as estimated by the difference between total amounts prior to precipitation and the remaining supernatant activities after antibody-precipitation.

Protein measurements. All protein measurements were performed after the method of Lowry (1951). Bovine serum albumin was used as standard.

Statistical analyses of the results. Analysis of the effects of different treatment upon kallikrein secretion [illegible] as evaluated by employing Wilcoxon [illegible] test for non-parametric data (van Eltéren 1960). The results presented in tables and figures are given as mean values ± the standard deviation.

Immunohistochemistry. Small pieces of the tissue were fixed *in vitro* in cold 96 [illegible] ethanol. However, glands stimulated by perfusion were fixed *in vivo* by infusing 96 [illegible] ethanol (3 ml/min for 3 min, 22°C) into the common carotid artery [illegible] hile cutting the jugular [illegible] to prevent an increase in intraglandular pressure. These glands were post-fixed in ethanol and then followed the usual schedule for tissue processing (Ørstavik [illegible] 1975). *In vivo* fixation [illegible] as impossible to perform on the control gland from the same animal, drug-[illegible] glands [illegible] therefore compared [illegible] with buffer-perfused and *in vivo*-fixed control glands from other [illegible] as well as [illegible] with the *in vitro*-fixed control pair gland.

Tissue sections (6 µm) were treated as described previously (Ørstavik *et al.* 1975). Kallikrein distribution [illegible] studied by [illegible] direct immunofluorescence technique employing [illegible] rhodamine conjugate of the immunoglobulin fraction of the above described anti-salivary kallikrein antiserum. The conjugate [illegible] as purified by [illegible] filtration and ion-exchange chromatography (Brandtzaeg 1973). The selected fraction had an optical [illegible] (OD) ratio ($OD_{[illegible]}/OD_{[illegible]}$) of 2.4:1 [illegible] was used at [illegible] concentration of 0.08 mg/ml and contained [illegible] precipitating unit per ml [illegible] hen tested against [illegible] standardized rat submandibular gland homogenate [illegible] 0.19 mg/ml kallikrein.

Drugs. Pentobarbital (Nembutal sodium [illegible] Abbott Laboratories, London); pilocarpine and epinephrine (AF [illegible] Oslo, Norway), acetylcholine chloride (F. A. F. Hoffmann-La Roche & Co. A. G.); norepinephrine [illegible] Norsk Astra AS, Oslo, Norway); isoproterenol (Isoprenaline sulphate [illegible] Norwegian medical [illegible]), phentolamine (Regitine [illegible] Ciba), propranolol chloride (Inderal [illegible] or Propranolol Hydrochloride [illegible] Imperial Chemical Industries Limited). Synthetic bradykinin was [illegible] gift from Sandoz, Switzerland.

Results

Bz-Arg-OEt-esterase activity as a parameter for glandular kallikrein. The monospecific sheep antiserum gave a normal precipitation curve for kallikrein (Fig. 1 A). At a dilution of 1 : 3 the maximal amount of protein was precipitated, and the Bz-Arg-OEt-esterase activity in the supernatant of the incubation mixture was completely neutralized (Fig. 1 A). When the antiserum concentration was kept constant (1 : 3) and the protein concentration of the added gland homogenate varied, a complete inhibition of both esterase and kinin-forming activity was observed when the antigen concentration was less than 0.7 mg wet gland [illegible] eight per ml (Fig. 1 B). Below this concentration of gland homogenate there was also [illegible] linear and parallel relationship between the increase in precipitated protein and precipitated esterase activity (Fig. 1 C). However [illegible] above this concentration some esterase activity reappeared, whereas kinin-forming activity was still completely neutralized (Fig. 1 B). This may be explained if the gland homogenate contained other esterases capable of hydrolyzing Bz-Arg-OEt than kallikrein or by the presence of a glandular inhibitor active against the kinin-forming activity but not against the esterase activity.

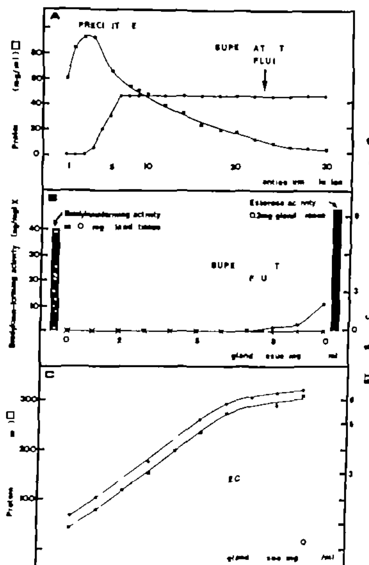


Fig. 1 The usefulness of the Bz Arg-OEt-esterase method f determination of submand bular gl kallikrein

A. The antiserum dilution that when added to 1 ml homogenized gland tissue containing 0 2 mg weight/ml, yielded the highest amount of precipitate, also removed all esterase activity from the sup natant fluid Thus, the monospecific anti kallikrei antiserum (100 μl) was ble to neutralize complet the esterolytic enzyme.

B Inh bition f bradyk nin-forming ability by the antiserum At high gland tissue concentrations so esterase activity reappeared while the antiserum still prevented ll kinin-formation Thi indicates presence of some non kallikrein esterase( ) or kininogenase inhibitors n the gland.

C. The protein content and esterolytic activity of the mmunoprecipitate showed a linea nd parl increase p to the gland tissue concentration of 0 7 mg/ml where both ki in formation and esterol activity were neutralized by the tiserum. I the expts. In Fig. 1 B nd C, 1 ml gland homogen containing 0–1.0 mg wet weight/ml was mixed with 100 μl antiserum d luted 1 3

As clearly demonstrated by the immunoelectrophoresis (Fig. 2), both the submandibul gland homogenate as well as sympathomimetically stimulated saliva contained except f kallikrein also the cross-reacting esterases A and B (Fig. 2) which are known to hydroly Bz Arg-OEt (Brandtzaeg *et al* 1976). However in the single radial immunodiffusion syster

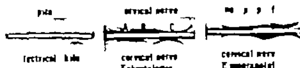


Fig. 2. Immunoelectrophoretic pattern of [illegible] saliva. Sympathomimetic stimulation of the gland resulted in massive secretion of kallikrein (C) as well as other trypsin-like esterases (esterase A and B, denoted A and B in the figure). The anti-kallikrein antiserum was raised against rat submandibular gland kallikrein and was not preabsorbed with the cross-reacting esterases *A and B* (see Methods). The various stimulation procedures are indicated in the figure.

the cross-reacting antigens did not give a precipitin ring even at very high concentrations. Furthermore, when added to the internal kallikrein standard, about 400 times higher concentration of esterases A and B than the applied kallikrein was necessary to increase the precipitin ring diameter 1.3 times. Brandtzaeg *et al.* (1976) showed that only 32% of the total gland Bz-Arg-OEt-esterase activity represented esterases A and B. Thus, the kallikrein antigenic activity measured represented kallikrein only. Estimates of gland kallikrein activity of the untreated control glands, measured by Bz-Arg-OEt-esterase and kallikrein antigenic activity showed good correlation (Table I). By relating the obtained kallikrein antigenic activities to specific esterase activity of purified submandibular gland kallikrein (fraction 4 C, Fig. 1 d, Table I, Brandtzaeg *et al.* 1976), the Bz-Arg-OEt-esterase activity caused by kallikrein could be calculated. Kallikrein esterase activity was 78 ± 16% of the measured total gland esterase activity. Also in saliva the two methods yielded comparable results, and

Table I. Comparison of wet weights, total protein contents, Bz-Arg-OEt-esterase and kallikrein antigenic activities of paired glands from 7 untreated control rats of the same age and sex. For the esterase activity the average percentage that G1 and G2 deviate from their mean value, was calculated. This percentage 5.2 ± 3.7 [illegible] then represents the methodological error of total gland esterase measurements if assuming that paired glands of the same animal are identical. The calculated data for G1 and G2 show that there are no statistically significant differences between paired glands. The standard deviations of the total means represent the biological variations from one animal to another and include the methodological errors. +G1 and G2 represent left and right gland respectively from the same animal. *protein, kallikrein antigenic and esterase activity [illegible] measured as described in Methods. $^{\S}p$ was calculated after Wilcoxon's test.

Rat	Wet weight g/gland		Total protein mg/gland		Total kallikrein antigenic activity mg/gland		Total Bz-Arg-OEt esterase EU/gland		deviation from mean esterase activity of G1&G2
	G1+	G2+	G1	G2	G1	G2	G1	G2	
1	0.245	0.248	33.8	34.2	3.07	3.20	2 973	2 944	0.5
2	0.196	0.198	35.3	35.4	2.54	2.68	2 315	2 089	5.1
3	0.244	0.218	22.0	26.7	1.97	2.29	1 497	1 822	9.8
4	0.196	0.193	23.9	23.9	1.93	1.85	1 001	1 237	10.5
5	0.254	0.253	33.4	37.2	3.10	3.12	2 910	3 049	2.3
6	0.195	0.196	27.4	25.6	2.85	2.65	1 710	1 831	3.4
7	0.196	0.203	25.6	25.8	2.53	2.71	1 919	2 117	4.9
G1≠G2, p	s.				n.s.		s.		
Total mean	0.217 ± 0.026		29.4 ± 5.1		2.60 ± 0.47		2 101 ± 682		5.2 ± 3.7

TABLE II Kallikrein secretio after stimulation of the rat submandibular gland. Kallikrein was quanta by its antigenic activity in a single radial immunodiffusion system and by its Bz Arg-OEt-este activity (see Methods). The secretion rate was expressed in esterase units (EU) per min.

Stimulation	Saliva				Gland
	Kallikrein antigenic activity mg/ml	Kallikrein flow rate EU/min	Total salivary kallikrein EU	n	Decrease in total gland esterase activity *EU*
Pilocarpine	0.06±0.05	0.4±0.2	17±14	3	146±314
Electrical hilus	0.15±0 08	2.0±1.0	38±11	5	280±120
Cervical nerve	30 9±11 6	136±90	800±414	5	1 056±733
Norepinephrine	*110.6±11* (23 7–272.5)	*304±255* (15–494)	*2 181± 145* (154–4 118)	*4*	*1 001±213*
Cervical nerve w/propranolol	51.2±61 8 (7 7 164)	111±161 (4–291)	031±2 967 (78–7 104)	7	757±364
Isoproterenol	1 7±1.8 (0.35–5.3)	1.2±1.2 (0.1 3.5)	29±29 (1 70)	6	8±333
Cervical nerve w/phentolamine	2.7±1.0 (1 7–3 9)	3 0±1 7 (1.5–5 7)	58±29 (39–102)	5	−158±373

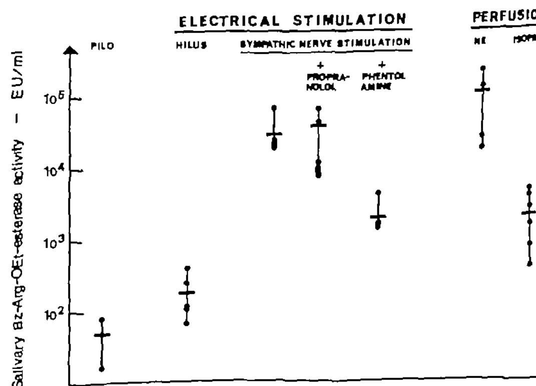


Fig. 3. Concentration of salivary kallikrein measured by its B Arg-OEt-esterase activity after stimulat of the gland The different sympathomimetic stimulation procedures were significantly (p 0 005) m effective the the parasympathom metic stimulation procedures i releasing kall krei into saliva. Al the effect of α adrenergic stimulation upon kallikrein secretion was significantly higher than β-adrener stimulation ($p < 0.005$).

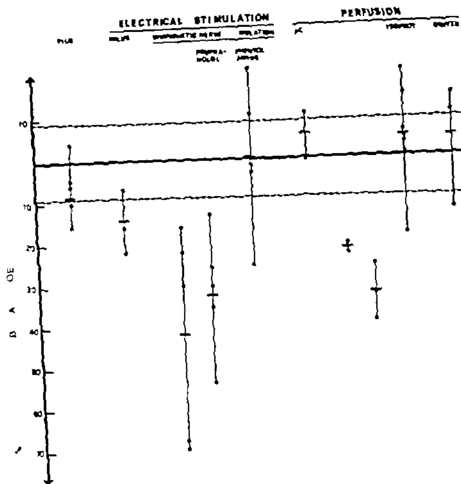


Fig. 4. Reduction in total Bz-Arg-OEt-esterase activity of the stimulated gland compared with the pair
control gland and expressed in percentage.
The dotted lines indicate the deviation of paired glands from untreated control rats from their tot
esterase activity (S ± 3.7).

no significant difference between the two estimates was detected after various stimulati- routes (Table III). Thus, variations in Bz-Arg-OEt-esterase activity clearly reflected quantit tive variations in kallikrein activity of the submandibular gland and its secretion.

Kallikrein activity of glands from untreated control rats. The two submandibular glan from one animal had equal wet weights, total protein content and Bz-Arg-OEt-esterase kallikrein antigenic activity whereas the differences between glands from different anim were considerable (Table I). Therefore, in the following expts. one of two paired glands used as control while the other was subjected to stimulation. No salivary secretion observed in unstimulated rats.

Kallikrein secretion after stimulation of the submandibular gland. Pilocarpine stimulat

TABLE II Kallikrein secretion after stimulation of the rat submandibular gland. Kallikrein was quantitated by its antigenic activity in a single radial immunodiffusion system and by its Bz-Arg-OEt-esterase activity (see Methods). The secretion rate was expressed in esterase units (EU) per min.

Stimulation	Saliva				Gland	
	Kallikrein antigenic activity mg/ml	Kallikrein flow rate EU/min	Total salivary kallikrein EU	n	Decrease in total gland esterase activity EU	
Pilocarpine	0.06±0.05	0.4±0.2	17±14	3	146±314	5
Electrical hilus	0.15±0.08	2.0±1.0	38±11	5	280±120	3
Cervical nerve	30.9±11.6	136±90	800±414	5	1 056±733	5
Norepinephrine	110.6±112 (23.7 272.5)	304±255 (15–494)	2 181±2 145 (154–4 118)	4	1 001±213	3
Cervical nerve w/propranolol	51.2±61.8 (7.7 164)	111±161 (4–291)	2 031±2 967 (78–7 104)	7	757±364	5
Isoproterenol	1.7±1.8 (0.35–5.3)	1.2±1.2 (0.1 3.5)	29±29 (1 70)	6	8±333	8
Cervical nerve w/phentolamine	2.7±1.0 (1.7 3.9)	3.0±1.7 (1.5–5.7)	58±29 (39 102)	5	−158±373	5

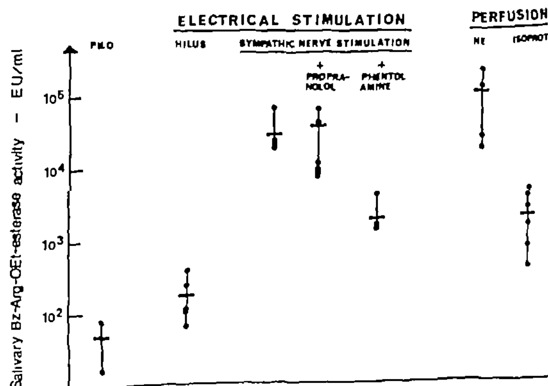
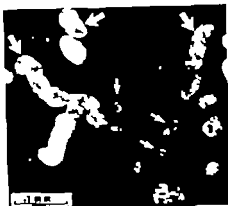


Fig. 3 Concentration of salivary kallikrein measured by its Bz-Arg-OEt-esterase activity after stimulation of the gland. The different sympathomimetic stimulation procedures were significantly ($p < 0.005$) more effective than the parasympathomimetic stimulation procedures in releasing kallikrein into saliva. Also the effect of α-adrenergic stimulation upon kallikrein secretion was significantly higher than β-adrenergic stimulation ($p < 0.005$).



ig. 5 The kallikrein distribution in an unstimulated submandibular gland detected by immunofluorescence (see Methods). Kallikrein is observed in the granular tubular cells (large arrows) and in the striated duct cells (small arrows).

y β-adrenergic stimulation contained about 45 times more kallikrein than did pilocarpine stimulated saliva (Table III). The amount of kallikrein secreted into saliva after -adrenergic stimulation varied considerably from one animal to another (7 810–165 200 EU/ml for cervical nerve stimulation with the addition of propranolol and 17 350–274 500 EU/ml after norepinephrine perfusion). For animals having the very high concentrations of salivary kallikrein the total amount of secreted enzyme greatly exceeded the measured decrease in total gland kallikrein. However after cervical nerve stimulation salivary kallikrein concentration varied from 17 933 to 60 500 EU/ml and the total amount of secreted enzyme corresponded to the decrease in gland kallikrein (Table II).

he results seemed to be largely independent on whether the salivary secretion was stimulated by drug perfusion or by electrical nerve stimulation. The perfusion procedure itself (tested by perfusing glands with buffer only) did not significantly alter the total esterase activity of the gland. Glands perfused with buffer contained 3 9 ± 16.2 % more esterase than r control glands, whereas the mean difference in esterase activity of paired untreated ntrol glands was 5.2 ± 3 7 % and never larger than 10.5 % (Table I). The wet weight of buffer-perfused glands was 30 ± 9 % higher than that of the control gland, most likely to accumulation of fluid as the perfused gland appeared edematous. Some salivary retion was observed in 2 out of 3 rats following buffer perfusion. The amount of kalli-in in these two secretions were 709 and 7 508 EU/ml. This was in contrast to glands not rfused or stimulated where no salivary secretion was detected. I p. injections of the anes-tics did not result in salivary secretion.

Immunohistochemical studies of the submandibular gland. The fluorescence antibody technique revealed no alteration in glandular kallikrein localization even after stimulation ocedures that from the above quantitative studies were known to activate kallikrein cretion (Fig. 5). The technique was, however not appropriate for detection of quantitative fferences in gland kallikrein content. Considerable amounts of kallikrei remained in the and even after massive kall krein secretion and variations in fluorescence intensity were fficult to detect. However by employing a conjugate dilution lower than the usual working lution (0.3 mg/ml protein) and close to the specific staining end point (0 08 mg/ml protein), e α-adrenergic induction of kallikrein release was substantiated by a decreased intensity

TABLE III Relative effect of different stimulation procedures on salivary kallikrein concentration. Kallikrein was measured by its antigenic activity in a single radial immunodiffusion system and by its Bz Arg-OEt-esterase activity (see Methods). The results are expressed in integers of the activity of pilocarpine stimulated saliva. The functional and immunological estimates showed corresponding variations.

Stimulation	Kallikrein antigenic activity	Bz Arg-OEt-esterase activity
Pilocarpine	1	1
Electrical hilus	3	4
Cervical nerve	515 ×	642 ×
Norepinephrine	1 843	2 375
Cervical nerve w/propranolol	853 ×	819
Isoproterenol	28 ×	44
Cervical nerve w/phentolamine	45	46

mandibular gland, 15 ± 10 µl/min and 9 ± 2 µl/min respectively. The amount of kallikrein secreted was small (Table II, Fig. 3), and there was no significant difference in esterase content between the stimulated and the control gland (Fig. 4).

Salivary flow following sympathetic nerve stimulation was 5 ± 2 µl/min. However, *sympathetic nerve stimulation increased kallikrein release significantly more* ($p < 0.01$) than did pilocarpine and gland hilus electrical stimulation. This stimulatory effect was reflected by measurements of total salivary kallikrein content (Table II), esterase activity (Fig. 3) and kallikrein antigenic activity (Table II) and also when expressed as reduction in total gland esterase activity (Fig. 4). The cervical sympathetic nerve stimulated saliva contained 642 times as much esterase and 515 times as much kallikrein antigenic activity as did pilocarpine stimulated saliva. Also, the total gland esterase activity was reduced by 42%, a reduction significantly greater than that obtained after pilocarpine stimulation ($p < 0.01$) and the differences between the paired glands of untreated control animals ($p < 0.005$). Epinephrine perfusion induced a 20% and 22% reduction in total esterase activity of the stimulated gland of the two animals tested.

The sympathetic release of kallikrein was predominantly mediated via α-adrenergic receptors since there was no significant difference between cervical nerve stimulation without and with the addition of a β-adrenergic blocker (propranolol) when total gland esterase activity was measured. Also, addition of an α-blocker (phentolamine) inhibited more than 93% ($p < 0.025$) of the effect of cervical nerve stimulation on kallikrein release (Fig. 3). Isoproterenol perfusion did not result in a significant decrease in total gland esterase, whereas norepinephrine perfusion caused 25%, 32% and 39% reduction of the enzyme in the three glands tested, all significantly greater than the observed differences in paired control glands. Furthermore, norepinephrine infusion and sympathetic nerve stimulation in the presence of propranolol resulted in about the same amount of salivary kallikrein as did cervical nerve stimulation alone. In contrast, isoproterenol perfusion and cervical nerve stimulation in the presence of an α-blocker (phentolamine) contained less than 3% of the amount of kallikrein observed in α-adrenergic induced saliva ($p < 0.01$) (Fig. 3, Table II). However, saliva induced

voluminous fluid secretion with low concentrations of kallikrein. In contrast, adrenergic stimulation resulted in lower salivary flow rates but with massive secretion of the enzyme as judged by the high salivary concentrations and decreased total gland esterase activities. The results may suggest an independent regulation of salivary flow rate and exocrine enzyme secretion. Also because kallikrein is exclusively a ductal protein, the above results indicate that at least the granular tubular cells and possibly also the striated duct cells receive a less extensive parasympathetic innervation.

The most efficient kallikrein secretion followed α-adrenergic stimulation, an observation which is in agreement with previous findings in the cat submandibular gland (Gautvik *et al.* 1974). There was considerable kallikrein secretion also after β-stimulation although significantly less than after α-stimulation. The doses of adrenergic blocking agents used were comparable to those used by others (Emmelin *et al.* 1965). However, also in the guinea pig a similar β-adrenergically induced release of submandibular gland kallikrein was observed using tissue cultures (Bhoola and Lemon 1975), and the norepinephrine stimulated enzyme secretion was only blocked after the administration of both propranolol and phentolamine (Albano *et al.* 1976).

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of cytoplasmic fluorescence of granular tubular cells in the stimulated gland as compared with the control gland The intensity of the fluorescence shown at this conjugate dilution was too low to permit microphotographic records.

Discussion

The present study shows that the Bz Arg-OEt-esterase method was highly suitable as a quantitative measure for the kallikrein content of the submandibular gland as well as kallikrein concentrations of saliva. The usefulness of this technique was ascertained by the parallel inhibitory effect of an anti-kallikrein antiserum on bradykinin forming ability and on Bz Arg-OEt-esterase activity Evidently not all esterolytic activity represented kallikrein since at high gland tissue concentrations some esterase activity remained in the supernatant fluid even though all kinin-forming activity was removed by the antibody precipitation. This agrees with the quantitation of kallikrein esterase activity which, by the use of the observed kallikrein antigenic activity was estimated to be as much as 78 ± 16% of the total gland esterase activity Also Brandtzaeg *et al* (1976) after purification of submandibular gland esterases found that 68% of total gland esterase represented kallikrein, and in addition, age dependent changes in gland esterase activity paralleled alterations in gland kallikrein antigenic activity Furthermore, in the present study changes in Bz Arg-OEt-esterase activity of saliva likewise paralleled changes in kallikrein antigenic activity

The various stimulation and perfusion procedures did not *per se* affect salivary and gland esterase activities. Observed differences between close arterial perfusion and non-perfusion stimulation (i.p drug administration and electrical nerve stimulation) were not significant. However perfusion with buffer alone provoked some salivary secretion in 2 out of 3 rats and the amount of kallikrein in these secretions showed large variations (709 and 7 508 EU/ml) This unexpected salivary kallikrein secretion may be due to destruction of glandular cells caused by the development of tissue edema. This assumption was based on the immunohistochemical observation of perfused glands where kallikrein occasionally was seen ubiquitously spread out over the entire tissue in parts of or in the entire gland This phenomenon was never observed in non-perfused glands In spite of development of tissue edema, the results from perfusion studies compared very well with the results obtained after cervical nerve stimulation. Also i p injections of liquid (anesthesia) did not provoke salivary secretion, thus the salivary flow induced by pilocarpine i p could not have been influenced by the drug solvent

The present study confirms and extends the previously documented (Brandtzaeg *et al* 1976, Ørstavik *et al* 1975 Ørstavik *et al* 1977) ductal localization of kallikrein. Kallikrein may at some stage during its synthesis or storage, be present in non-ductal cells in an immunochemically different form. However physiological activation of the gland should make kallikrein to appear and thus disclose its true site of synthesis. No change in kallikrein localization was observed during the different experimental conditions.

Parasympathomimetic and adrenergic stimulation was always followed by saliva formation and kallikrein secretion although salivary flow rate and enzyme release varied to a large extent Like in other species (Gautvik *et al* 1974) parasympathomimetic stimulation lead

to voluminous fluid secretion with low concentrations of kallikrein. In contrast, adrenergic stimulation resulted in lower salivary flow rates but with massive secretion of the enzyme as judged by the high salivary concentrations and decreased total gland esterase activities. The results may suggest an independent regulation of salivary flow rate and exocrine enzyme secretion. Also, because kallikrein is exclusively a ductal protein, the above results indicate that at least the granular tubular cells and possibly also the striated duct cells receive a less extensive parasympathetic innervation.

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We are grateful to Dr. Kjell Nustad for the supply of purified submandibular gland esterases and antisera against kallikrein.

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Acta physiol. scand. 1977 100 45–50

From the Department of Physiology I, Karolinska Institutet, Stockholm, Sweden

Deuterium Induced Extinction of ADH-release in Response to Intracerebroventricular Infusions of Hypertonic NaCl and Angiotensin

By

M. Rundgren, L. G. Leksell, F. Lishajko and B. Andersson

Received 7 October 1976

Abstract

Rundgren, M., L. G. Leksell, F. Lishajko and B. Andersson. *Deuterium induced extinction of ADH-release in response to intracerebroventricular infusions of hypertonic NaCl and angiotensin.* Acta physiol. scand. 1977 *100* 45–50

Infusions (20 µl/min) of hypertonic (0.3 M) NaCl and angiotensin II (1 µg/kg min^{-1}) in isotonic (0.15 M) NaCl were made for 1 h in the hydrated goat during fully developed water diuresis. Either H_2O or deuterium (D_2O) was used as solvent. A pronounced antidiuretic response, outlasting the infusion period by 30 min or more, was seen when the substances were dissolved in H_2O. Only a slight inhibition of the water diuresis, which was extinguished during the infusion period, was obtained when D_2O was used as the solvent. The infusion of 0.3 M NaCl/H_2O invariably induced drinking in one of the goats, which, however, showed no drinking response to the infusions of 0.3 M NaCl/D_2O. The possibility is discussed that D_2O (perhaps by its inhibitory effect on (Na^+-K^+)-ATPase activity) reduced the sensitivity of juxtaventricular receptors regulating ADH-release and water intake.

The integrated results of studies performed over the past several years in the goat imply that tasks in the cerebral control of water balance which hitherto have been attributed to hypothalamic osmoreceptors (Verney 1947) instead are executed by juxtaventricular sodium sensitive receptors (*cf.* Andersson 1974). Among the supports for this idea are the observations that intracerebroventricular (IVT) infusions of iso- or hypertonic saccharide solutions inhibit the basic secretion of antidiuretic hormone (ADH) in non-hydrated normovolemic animals (Eriksson 1974), attenuate dehydrative drinking (Olsson 1975), and repress the dipsogenic and antidiuretic responses to intracarotid infusions of hypertonic NaCl (Olsson 1973). The IVT infusions of pure saccharide solutions appear to attenuate drinking and ADH-release primarily by dilution-reduction of cerebrospinal fluid (CSF) [Na^+] since no, or considerably less inhibitory effect has been obtained with saccharides dissolved in isotonic saline. However, influence of the carbohydrates on choroidal and/or periventricular enzymatic Na^+ transport may also have to be considered in view of some recent studies. Glycerol inhibits (Na^+-K^+)-ATPase (Mayer and Avi-Dor 1970), and IVT infusions of iso- or

hypertonic solutions of glycerol have been found to inhibit the ADH-release and exting dehydrative thirst much more effectively than corresponding infusions of equi-osm glucose solutions (Olsson 1976, Olsson, Larsson and Liljekvist 1976) Futhermore, ano inhibitor of (Na^+–K^+)-ATPase, deuterium (D_2O) (Ahmed and Foster 1974), has the s effect on the water balance as glycerol when administered into the lateral cerebral vent with NaCl added to isotonicity (Leksell, Lishajko and Rundgren 1976) These observat vaguely indicate that (Na –K)-ATPase activity at some stage is essential for the excita of juxtaventricular receptors regulating ADH release and water intake. If so the IVT ministration of D O would be expected to counteract also ADH release and thirst in sponse to IVT infusions of hypertonic NaCl This possibility has been tested in the pre study

The ADH-releasing and dipsogenic effects of IVT infusions of angiotensin II have b found to be highly dependent upon the [Na] of the CSF This has led to the suggestion angiotensin in these respects may act by lowering the stimulus threshold of juxtaventric receptors which primarily are sodium sensitive (Andersson 1971). Therefore, it was of int to extend this study to involve also IVT infusions of angiotensin dissolved in D_2O and NaCl added to isotonicity

Methods

Animals. 2 adult female goats (pre-hydration b.wt 31 and 36 kg) were used The animals were ke metabolism cages where all expts. were conducted, and where the goats had free access t hay and w The animals were maintained i positive sodium balance by receiving 6 g of NaCl added to a daily rati 300 g commercial grain mix.

IVT implantations and infusions The goats had a 3-cannulae system (Åkerlund, Andersson and Ol 1973) permanently implanted into ne lateral cerebral ventricle with the ventricular outlet near the fora of Monro The outer and middle cannulae were made of platinum–iridium order to minimize for body reaction. Free communication with the CSF was ensured before the start of and after each infusio by observing drainage of CSF through the middle cannula on compression of the jugular vein Infusions were started round 10 a.m and the minimum time interval between each expt w 2 days d ration of the infusions was 1 h with a rat of 20 µl/min

The following solutions were infused into the lateral ventricle 0.3 M NaCl in either H O or D_2O (' ma), and angiotensin II (Hypertensin Ciba®) in 0.15 M NaCl with either H O or D_2O as the solv The amount of giotensi administered was 1 ng/kg min^{-1}

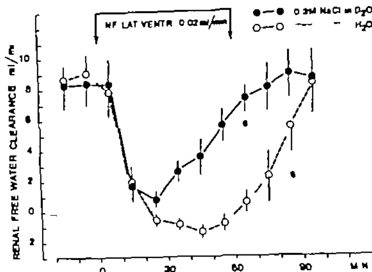
Hydration. All expts. were made in the hydrated animal when a water diuresis was fully establis Hydration was accomplished by giving i to the rumen by stomach tube 100 ml/kg of 38°C water a 80 min bef re the IVT infusion was tarted

Urine collection and analyse Urine was collected in 10 min samples via a etention catheter inserted the urinary bladder Urine [Na] and [K] were determined by use of an IL 343 flame photometer "Advanced Instruments Inc. osmometer was used fo measuri g rine and blood plasma osmolali plasma osmolal ty round 290 mosm/kg was found during hydration. Hence this value was used for culations of the renal free water clearance (C_{H_2O}).

Results

ADH release

Response to hypertonic NaCl As shown in Fig. 1 (*circles*) the IVT infusion of 0.3 M N dissolved in H_2O (n = 6) effectively inhibited the water diuresis and induced negative re



g. 1. Deuterium (D_2O) reduced extraction of the antidiuretic response to the intracerebroventricular ʼusion of hypertonic NaCl in the hydrated goat. Symbols represent mean and the vertical bars S.E. No rrection is made for the dead-space of the catheter and tubing used for collection of urine.

eriod, and the water diuresis did not return to pre-infusion intensity until about 40 min ater. The corresponding IVT administration of hypertonic NaCl dissolved in D_2O (n 6) lso initially induced an inhibition of the water diuresis (Fig. 1 *dots*). However C_{H_2O} did ot become negative. It started to rise again after about 30 min to reach the high initial evel in the first post-infusion period of urine collection.

Response to angiotensin II The antidiuretic response to the IVT infusion of angiotensin n isotonic NaCl/H_2O solution (n 6) was less pronounced than the response to the IVT nfusion of 0.3 M NaCl in H_2O. Negative C_{H_2O} did not develop, but the water diuresis re mained considerably inhibited throughout the infusion period and for at least 30 min after wards (Fig. 2, *open triangles*). Initial inhibition of the water diuresis was also observed during the IVT infusion of angiotensin in isotonic NaCl/D_2O (n = 6), but now C_{H_2O} started to rise again after 30 min and reached pre-infusion level in the first or second post-infusion period of urine collection (Fig. 2, *filled triangles*).

Water intake and renal salt excretion

In spite of being hydrated, one of the goats invariably responded with drinking to the IVT infusion of 0.3 M NaCl in H_2O. Drinking commenced between 10 and 30 min after the start of the IVT infusion (3), and 250 to 500 ml of water was consumed cumulatively during the remainder of the infusion period. In contrast, the IVT infusion of hypertonic NaCl in D_2O (3) did not induce water intake in this goat. The other animal did not drink in response to any of the infusions of hypertonic NaCl, and none of the goats drank in response to the angiotensin infusions.

Both the infusion of hypertonic NaCl and of angiotensin caused an increase in renal Na

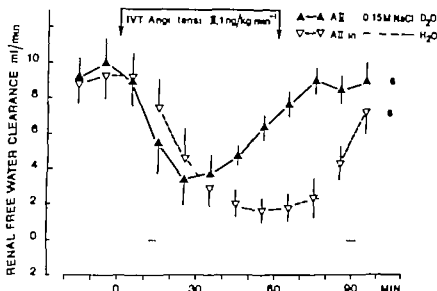


Fig. 2. Deuterium (D_2O) induced extinction of the antidiuretic response to the intracerebroventricular infusion of angiotensin II in the hydrated goat. Rate of infusion = 20 µl/min. Symbols represent means and the vertical bars S.E. No correction made for the dead-space of the catheter and the tubing used for urine collection.

and K excretion. However, no significant difference in the saluretic response could be observed between the H_2O and the D_2O experiments.

Discussion

The inhibition of the water diuresis of goats which is obtained in response to IVT infusion of hypertonic NaCl and angiotensin is due to neurohypophyseal release of ADH. This has been demonstrated in previous expts. involving interruption of the neural connection between the hypothalamus and the pituitary (Andersson, Dallman and Olsson 1969, Andersson *et al.* 1972) and bioassays of urinary ADH activity (Lishajko and Andersson 1975). The present study has revealed that these stimuli of ADH release gradually can be made ineffective by the concomitant IVT administration of D_2O, and indicates that this holds true also for hypertonic NaCl as IVT stimulus of thirst. An explanation may be that deuterium infused into the cerebroventricular system reduces the sensitivity of cerebral receptors regulating ADH release and water intake. It has been shown (Bering 1952) that D_2O injected into the blood very rapidly attains equilibrium with the extra- and intracellular brain tissue water, and that deuterium in the CSF is continually exchanging at all surfaces of the brain by free diffusion. Similar distribution studies with radioactive water have revealed that approximately 90% of it is extracted from the blood to brain during a single capillary transit (Eichling *et al.* 1974). When perfused through the cerebroventricular system at a constant rate, labelled water and other substances with almost unlimited passage over the blood-brain barrier attain a very steep CSF/brain-tissue concentration profile (Patlak and Fenster

macher 1975). It means that the brain tissue concentration of D_2O in the present expts. by all probability remained negligible at about 1 mm from the ventricular walls. It seems to exclude a direct inhibitory action of D_2O on receptors located at any further distance from the ventricles. However the effects of medially placed forebrain lesions in the goat indicate that the cerebral receptors which regulate ADH-release and water intake predominantly are located in, or very close to the anterior wall of the third ventricle (Andersson, Leksell and Lishajko 1975). This juxtaventricular area seems to have been exposed to a relatively high concentration of D_2O since the infusions were made into the lateral ventricle near the foramen of Monro. It is impossible, however to judge whether the concentration of D_2O in this area ever became sufficient for deuterium to exert its known inhibitory effect on enzymatic Na^+ transport (Ahmed and Foster 1974).

The suggestion that the degree of excitation of a juxtaventricular sensory mechanism regulating ADH-release and thirst may be determined by the local (Na^+–K^+)-ATPase activity cautiously has been put forward as a working-hypothesis in consequence of effects on water balance observed during IVT infusions of glycerol (Olsson *et al* 1976) and D_2O (Leksell *et al* 1976). The present expts. lend further support to the hypothesis, as do earlier studies of the effects of hypothalamic ouabain implants in the rat (Bergmann *et al* 1967 Gutman, Bergmann and Zerachia 1971)

As regards IVT infusion of glycerol, another explanation for its exceptional efficiency to inhibit ADH-release and thirst has also to be considered. Even when dissolved in isotonic NaCl, glycerol causes a considerable reduction of CSF [Na^+] which could well be the main cause of its effect on the water balance (Olsson *et al.* 1976). CSF [Na^+] was not measured during, or after the IVT infusions of deuterium. However that the IVT administration of D_2O with NaCl added to hypertonicity should cause lowering of the prevailing CSF [Na^+] appears unlikely since extraction of deuterium from brain to blood takes place extremely rapidly and much faster than the extraction of glycerol.

The fact that D_2O extinguished also ADH-release in response to IVT infusions of angiotensin supports the idea that elevated CSF [Na^+] and angiotensin affect water balance via the same receptors (Andersson 1971). The effects of experimentally induced alterations in CSF [Na^+] and IVT infusions of angiotensin suggest that a periventricular sodium and angiotensin sensitive mechanism also is involved in the regulation of renal Na^+ excretion (Andersson 1974). However in the present expts. deuterium was not found to reduce the natriuretic response to IVT infusions of hypertonic NaCl and angiotensin. The reason could be that cerebral receptors involved in the control of sodium balance predominantly are present more posterior along the cerebroventricular system than those regulating ADH-secretion and water intake. This is indicated by the recent observation (Passo, Thornborough and Rothballer 1975, Thornborough and Passo 1975) that local elevation of the CSF [Na^+] in the fourth ventricle induces natriuresis. Due to rapid diffusion out of the lateral and third ventricles and fast extraction by the blood, no, or very little D_2O may have reached as far posterior as to the fourth ventricle in the experiments reported here.

This work was supported by the Swedish Medical Research Council (project 04X-00503) and by grants from Karolinska Institutet. The generous gift of Hypertensin from the Hässle-Ciba-Geigy Company is gratefully acknowledged.

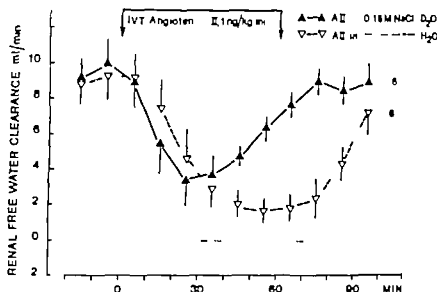


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Acta physiol. scand. 1977 100 51–55

From the Department of Pharmacology University of Göteborg, Sweden

Shortlasting Increase in the Synthesis and Utilization of Noradrenaline due to Axotomy Induced Irritation

By

NILS-ERIK ANDÉN

Received 11 October 1976

Abstract

ANDÉN, N. E. *Shortlasting increase in the synthesis and utilization of noradrenaline due to axotomy-induced irritation.* Acta physiol. scand. 1977 *100* 51 55

The synthesis and the utilization of noradrenaline in the caudal and cranial part of intact and sectioned rat spinal cords were determined at 20 h or for 30 min 2 h after an operation. The synthesis and the utilization of noradrenaline in the caudal part of spinal cords transected 20 h previously were retarded as compared to those in the cranial part of sectioned cords or in the caudal part of intact cords. The synthesis of noradrenaline was stimulated in the caudal and cranial part of the spinal cord when measured for 30 min after transection. The utilization of noradrenaline in the spinal cord caudal to transection was not decelerated during h after an operation. Local application of lidocaine to the cord prior to the cut changed the synthesis and the utilization of noradrenaline in both parts of the spinal cord to values similar to those obtained 20 h after the operation. Transection of the spinal cord might stimulate the synthesis and the utilization of noradrenaline by shortlasting mechanical irritation of neurons cut by the lesion

Key words: Noradrenaline, synthesis, utilization, spinal cord, axotomy local anesthesia

he noradrenaline (NA) in the spinal cord occurs in nerve terminals of neurons, the cell odies of hich are present in the lower brain stem (Carlsson *et al.* 1964, Andén *et al* 1964 hlström and Fuxe 1965) Therefore, a transection of the spinal cord should interrupt the erve impulse flow to the NA nerve terminals in the cord caudal, but not cranial to the sion. The NA in the spinal cord caudal to a section starts to disappear only after more han 4 h indicating that there is no degeneration during the first day (Andén *et al* 1964, Magnusson 1973 Grabowska and Andén 1976). The disappearance of the NA in the spinal cord following inhibition of the NA synthesis is markedly retarded caudal to an acute transection, as compared to the cranial part of sectioned cords or to the caudal part of intact cords (Andén *et al* 1966, Andén, Fuxe and Hökfelt 1966, Andén, Fuxe and Hökfelt 1967 Andén and Fuxe 1971 Grabowska and Andén 1976). In an analogous way the synthesis of NA has also been found to be dependent on nerve impulses when determined as the accumulation of Dopa following Dopa decarboxylase inhibition (Grabowska and Andén 1976). In these studies, the synthesis inhibitors have been given more than 1 h after the operation hen the animals have recovered from the ether or barbiturate anesthesia. There are indi-

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...LE II Concentration of noradrenaline in the cranial and caudal parts of the rat spinal cord intact or sectioned at Th 4 *in vivo* The rats were operated on 20 h, h or 1 h before sacrifice. Lidocaine (0.1 ml of 2 solution) as applied to the Th 4 region of the spinal cord 1.1 min before sacrifice. *DL*-α-methyltyrosine methylester (α-MT 250 mg/kg i p) was given 2 h or 1 h before sacrifice. The values are means ith the number of expts. in parentheses. The values are expressed as per cent of intact controls operated on 20 h before sacrifice (cranial part = 0.29 µg/g, caudal part 0 40 µg/g). Statistical significances were calculated by one-way analysis of variance followed by test (F 1 6.5, variance within groups 202.909 *df* ... groups = 243).

...me between ...eration and ...rifice	Drug treatment	Intact cord		Sectioned cord	
		Cranial part	Caudal part	Cranial part	Caudal part
0 h	No drugs	100.0 (8)	100.0 (8)	80.7 (8)	95.6 (8)
1 h	No drugs	96.0 (8)	99.1 (8)	92.9 (8)	103.5 (8)
2 h	No drugs	89.9 (9)	98.8 (9)	80.6 (8)	111.1 (8)
2 h	Lidocaine	100.7 (8)	105.2 (8)	100.7 (8)	117.6 (8)
0 h	α-MT 1 h	67.7 (8)	69.5 (8)	70.1 (8)	87.7 (8)
0 h	-MT 2 h	58.9 (8)	68.3 (8)	52.9 (7)	82.1 (7)
1 h	α-MT 1 h	72.1 (7)	73.5 (7)	65.3 (7)	74.7 (7)
2 h	α-MT 2 h	59.8 (7)	54.7 (7)	49.4 (7)	64.1 (7)
2 h	Lidocaine α-MT 2 h	67.1 (8)	72.2 (8)	60.4 (8)	88.2 (8)

Differences necessary for

p 0.001 22.3 (9 9), 23.0 (9 + 8), 23.7 (8 8), 23.9 (9 7), 24.5 (8 7), 25.3 (7 7)
p 0.01 17.3 (9 9), 17.9 (9 8), 18.4 (8 8), 18.5 (9 + 7), 19.0 (8 + 7), 19.7 (7 7),
p 0.05 13.2 (9 9), 13.6 (9 8), 14.0 (8 8), 14.1 (9 7), 14.5 (8 7), 15.0 (7 7)

department), *DL*-α-methyltyrosine methylester HCl (α-MT H 44 68 generously donated by Hässle Ltd Mölndal), lidocaine HCl (2 solution, Xylocain Astra, Södertälje), halothane (Fluothane ICI Ltd Alderley Park, Macclesfield) All the doses refer to the forms indicated above

Results

Synthesis of Noradrenaline

The synthesis of NA was determined as the accumulation of Dopa induced by NSD 1015 and it was compared with that in the cranial and caudal parts of intact spinal cords of rats operated on 20 h prior to sacrifice (T ble I). A transection of the spinal cord 20 h prior to sacrifice reduced the synthesis of NA in the caudal (p 0.05), but not in the cranial part.

An acute section of the spinal cord stimulated the synthesis of NA to about the same marked degree in the caudal and in the cranial part (Table I). The increases in the synthesis of NA were much smaller following an acute operation without lesion of the spinal cord. Application of the local anesthetic agent lidocaine to the spinal cord almost completely inhibited the acute effects of lesion on the synthesis of NA.

Utilization of Noradrenaline

In the caudal part of the spinal cord sectioned 20 h before sacrifice, the endogenous NA concentration was not changed (Table II). The α-MT-induced disappearance of NA was retarded in the caudal part, i.e the concentration of NA was significantly (p < 0 001) higher in the caudal than in the cranial part 2 h after the administration of MT and 20 h after a transection.

TABLE I Accumulation of Dopa induced by 3-hydroxybenzylhydrazine (NSD 1015 100 mg/kg i p 30 min before sacrifice) in the cranial and caudal parts of the rat spinal cord i tact or sectioned at Th 4 *in vivo* The rats were operated on 20 h or 30 min before sacrifice Lidocaine (0.1 ml of a 2 % solution) was applied to the Th 4 region of the spinal cord 31 min before sacrifice. The values are means with the number of expts. in parentheses. The values are expressed as per cent of intact controls operated on 20 h before sacrifice (cranial part = 0.053 μg/g, caudal part 0.056 μg/g). Statistical significances were calculated by one-way analysis of variance followed by *t*-test ($F = 13.686$, variance within groups = 1 033 71 *df* within groups = 103).

Time between operation and sacrifice	Drug treatment	Intact cord		Sectioned cord	
		Cranial part	Caudal part	Cranial part	Caudal part
20 h	NSD	100.0 (10)	100 0 (10)	95.3 (10)	63.1 (10)
30 min	NSD	135.3 (10)	138.6 (10)	193.8 (10)	183 5 (10)
30 min	Lidocaine + NSD	112.2 (9)	86.4 (9)	120 9 (9)	100 6 (9)

Differences necessary for:

$p < 0\ 001$: 48.7 (10 + 10), 50 0 (10 + 9), 51.3 (9 + 9)
$p < 0.01$ 37 7 (10 + 10), 38.8 (10 + 9), 39.8 (9 + 9)
$p < 0\ 05$ 28.5 (10 + 10), 29.3 (10 + 9), 30.0 (9 + 9).

cations, however that the disappearance of the NA following synthesis inhibition is of about the same magnitude cranial and caudal to a transection of a spinal cord during a period of 4 h immediately after a lesion (Andén *et al* 1966) These studies have prompted the present investigations of the changes in the synthesis and utilization of NA in the spinal cord at short intervals after a transection and how these changes are produced.

Materials and methods

Male Sprague-Dawley rats weighing 155–240 g were used. All the rats were operated on during halothane anesthesia. In this way only a short anesthesia was needed. After midline incisions in the sk and the muscles, the spinous process of Th 3 was removed by means of a forceps and a small pa r of scissors, so that the segment Th 4 of the spinal cord was exposed. I the lidocaine expts., bout 0.1 ml of a 2 solution was applied to the pinal cord about 1 min prior to the imagined or real transection. I half of the rats the spinal cord was transected by means of small pa of scissors. Great care wa taken to void damage of the spinal cords of the intact groups After suturing of the skin, the anesthesia was d scontinued and the rats wok up within 1 2 m n. The rectal temperature was frequently mon tored and the ambient temperature was changed, if necessary

Biochemistry The synthesis of NA was determined by the ccumulation of Dopa during 30 min following the dministration of the Dopa decarboxylase inhibitor 3-hydroxybenzylhydrazine HCl (100 mg/kg i p NSD 1015) (Carlsson *et al* 1972 a). The utilization of NA was determined by the d sappearance of NA during 1 or 2 h followi g the administration of the tyrosine hydroxylase nh bitor α methyltyrosine (250 mg/kg i.p of the *DL*-f rm of the methylester HCl α MT) (Spector *et al* 1965 Corrod and Hanson 1966, Andé *et al.* 1966). The NSD 1015 nd the α MT were given intraperitoneally less than 1 min before the transection.

The rat were killed by thoracotomy and exsanguination d ring light chloroform nesthesia The spinal cords were taken out as quickly as possible nd the roots and meninges were removed After the transection described bove, the cranial and the caudal parts of the pinal cords had appro mately the same weight. The spinal cords of the intact groups were divided postmortally in a similar ma ner I all expts., the parts of the spinal cords from two rats were pooled. The Dopa and the NA were determined spectrofluorimetrically after homogenization in 0.4 N perchloric acid, cation exchange chromat graphy o idation and rearrangement (Atack and Magnusson 1970, Kehr Carlsson and Lindqvist 1972, Häggendal 1963)

Drugs The following drugs were used 3-hydroxybenzylhydrazine HCl (NSD 1015 synthesized in this

TABLE II. Concentration of noradrenaline in the cranial and caudal parts of the rat spinal cord intact or sectioned at Th 4 in rats. The rats were operated on 20 h, 2 h or 1 h before sacrifice. Lidocaine (0.1 ml of 2 % solution) was applied to the Th 4 region of the spinal cord 1 min before sacrifice. DL-α-methyltyrosine methylester (α-MT 250 mg/kg i.p.) was given 2 h or 1 h before sacrifice. The values are means with the number of expts. in parentheses. The values are expressed as per cent of intact controls operated on 20 h before sacrifice (cranial part 0.29 μg/g, caudal part 0.40 μg/g). Statistical significances were calculated by one-way analysis of variance followed by t-test (F 12.635, variance within groups 202.909, df within groups = 243).

Time between operation and sacrifice	Drug treatment	Intact cord		Sectioned cord	
		Cranial part	Caudal part	Cranial part	Caudal part
20 h	No drugs	100.0 (8)	100.0 (8)	80.7 (8)	95.6 (8)
1 h	No drugs	96.0 (8)	99.1 (8)	93.9 (8)	103.5 (8)
2 h	No drugs	89.9 (9)	98.8 (9)	80.6 (8)	113.1 (8)
2 h	*Lidocaine*	*100.7 (8)*	*105.2 (8)*	*100.7 (8)*	*117.6 (8)*
20 h	α-MT 1 h	67.7 (8)	69.5 (8)	70.1 (8)	82.7 (8)
20 h	α-MT 2 h	58.9 (8)	68.3 (8)	5 9 (7)	82.1 (7)
1 h	α-MT 1 h	72.1 (7)	73.5 (7)	65.3 (7)	74.7 (7)
2 h	*α-MT 2 h*	*59.8 (7)*	*56.7 (7)*	*49.4 (7)*	*64.1 (7)*
2 h	Lidocaine α-MT 2 h	67.1 (8)	72.2 (8)	60.4 (8)	88.2 (8)

Differences necessary for

p 0.001 22.3 (9 9), 23.0 (9 8), 23.7 (8 8), 23.9 (9 7), 24.5 (8 7), 25.3 (7 + 7)
p 0.01 17.3 (9 9), 17.9 (9 8), 18.4 (8 8), 18.5 (9 7), 19.0 (8 7), 19.7 (7 7);
p 0.05 13.2 (9 9), 13.6 (9 8), 14.0 (8 8), 14.1 (9 7), 14.5 (8 7), 15.0 (7 7).

department), DL-α-methyltyrosine methylester HCl (α-MT H 44/68 generously donated by Hässle Ltd Mölndal), lidocaine HCl (2 solution, Xylocain Astra, Södertälje), halothane (Fluothane ICI Ltd Alderley Park, Macclesfield). All the doses refer to the forms indicated above.

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Time between operation and sacrifice	Drug treatment	Intact cord		Sectioned cord	
		Cranial part	*Caudal part*	*Cranial part*	*Caudal part*
20 h	NSD	100.0 (10)	100.0 (10)	95.3 (10)	63.1 (10)
30 min	NSD	135.3 (10)	138.6 (10)	193.8 (10)	183.5 (10)
30 min	Lidocaine + NSD	112.2 (9)	86.4 (9)	120.9 (9)	100.6 (9)

Differences necessary for

p < 0.001 48.7 (10 + 10), 50.0 (10 + 9), 51.3 (9 + 9)
p < 0.01 37.7 (10 + 10), 38.8 (10 + 9), 39.8 (9 + 9)
p < 0.05 28.5 (10 + 10), 29.3 (10 + 9), 30.0 (9 + 9).

cations, however that the disappearance of the NA following synthesis inhibition is of about the same magnitude cranial and caudal to a transection of a spinal cord during a period of 4 h immediately after a lesion (Andén *et al* 1966). These studies have prompted the present investigations of the changes in the synthesis and utilization of NA in the spinal cord at short intervals after a transection and how these changes are produced.

Materials and methods

Male Sprague-Dawley rats weighing 155–240 g were used. All the rats were operated on during halothane anesthesia. In this way only a short anesthesia was needed. After midline incisions of the skin and the muscles, the spinous process of Th 3 was removed by means of a forceps and a small pair of scissors, so that the segment Th 4 of the spinal cord was exposed. In the lidocaine expts., about 0.1 ml of a 2% solution was applied to the spinal cord about 1 min prior to the imagined or real transection. In half of the rats, the spinal cord was transected by means of a small pair of scissors. Great care was taken to avoid damage of the spinal cords of the intact groups. After suturing of the skin, the anesthesia was discontinued and the rats woke up within 1–2 min. The rectal temperature was frequently monitored and the ambient temperature was changed if necessary.

Biochemistry. The synthesis of NA was determined by the accumulation of Dopa during 30 min following the administration of the Dopa decarboxylase inhibitor 3-hydroxybenzylhydrazine HCl (100 mg/kg i.p. NSD 1015) (Carlsson *et al* 1972). The utilization of NA was determined by the disappearance of NA during 1 or 2 h following the administration of the tyrosine hydroxylase inhibitor α-methyltyrosine (250 mg/kg i.p. of the *DL* form of the methylester HCl α MT) (Spector *et al* 1965, Corrodi and Hanson 1966, Andén *et al.* 1966). The NSD 1015 and the α MT were given intraperitoneally less than 1 min before the transection.

The rats were killed by thoracotomy and exsanguination during light chloroform anesthesia. The spinal cords were taken out as quickly as possible and the roots and meninges were removed. After the transection described above, the cranial and the caudal parts of the spinal cords had approximately the same weight. The spinal cords of the intact groups were divided postmortally in a similar manner. In all expts., the parts of the spinal cords from two rats were pooled. The Dopa and the NA were determined spectrofluorimetrically after homogenization in 0.4 N perchloric acid, cation exchange chromatography, oxidation and rearrangement (Atack and Magnusson 1970, Kehr, Carlsson and Lindqvist 1972, Häggendal 1963).

Drugs. The following drugs were used: 3-hydroxybenzylhydrazine HCl (NSD 1015, synthesized in this

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The endogenous concentrations of NA were not markedly altered after an acute operation or section though there was a tendency to an elevation in the caudal part of the sectioned cords (Table II). The α MT-induced disappearance of NA in the caudal part of the spinal cord was of almost the same percentage in the rats with an acute section of the spinal cord as in those with only an acute operation. The NA in the caudal part of the sectioned cords 2 h after α MT was significantly ($p < 0.05$) lower when the lesion had been performed 2 h rather than 20 h before sacrifice. Local application of lidocaine to the spinal cord prior to section completely antagonized the lesion-induced acceleration of the disappearance of NA 2 h after α-MT, *i.e.*, the NA was significantly ($p < 0.01$) higher in the caudal part of the sectioned cords following pretreatment with lidocaine.

Discussion

The synthesis and the utilization of NA in the caudal part of the sectioned cord were accelerated if the operation was carried out acutely (30 min–2 h) as compared to 20 h prior to sacrifice. Since the synthesis and the utilization were changed similarly at all intervals, the endogenous concentration of NA was not markedly influenced. The acute effects on the synthesis and the utilization of NA following the axotomy were almost completely inhibited by local pretreatment with a local anesthetic. Therefore it is likely that the section produced excitation of the cut nerve fibres, perhaps due to the mechanical irritation. Such a stimulation might influence the descending NA neurons to the caudal part of the spinal cord, but also other descending and ascending neurons in all probability. The increased synthesis of NA in the cranial part of the acutely sectioned cords should be mediated from the lesion via neurons not containing NA.

An acute axotomy of the ascending NA neurons to the neocortex by a cerebral hemisection appears to lead to changes similar to those in the spinal cord: during the 30 min after the operation the synthesis of NA is usually slightly elevated and the disappearance of NA following the Dopa decarboxylase inhibition is usually somewhat accelerated (Carlsson *et al.* 1972 b, Kehr 1974, Kehr, Carlsson and Lindqvist 1976). The changes in the turnover of NA are different from those reported after an acute axotomy of the ascending DA neurons by a cerebral hemisection or an electrolytic lesion: the concentration of DA is rapidly elevated due to a marked stimulation of the synthesis of DA simultaneously with a retardation of the utilization of DA (Andén *et al.* 1972, Kehr *et al.* 1972, Stock, Magnusson and Andén 1973, Walters, Roth and Aghajanian 1975). Apparently there is a receptor-mediated increase in the synthesis of DA, but not in that of NA, in the absence of nerve impulses. The reason for this difference between DA and NA neurons is unknown at present. It also appears that the NA neurons are more stimulated than the DA neurons by the axotomy-induced mechanical lesion.

This work was supported by the Swedish Medical Research Council (04X 50). Excellent technical assistance was given by Mrs Inger Oscarsson.

state with urea as denaturant. This normally takes place at urea concentrations of 6–8 M and the denaturated proteins exist as random coils (Tanford 1968). Other work has shown that proteins (.g enzymes) can undergo functional changes in low concentrations of urea (2 M) (Takashima and Kasuya 1967 Soape *et al* 1974) and studies of hemoglobin have shown a decreased cooperation between the subunits, and a concomitant increase in O_2-affinity for hemoglobin at low urea concentrations (Sharma, E erse and Ramsey 1975). Also, low concentrations of urea enhance α-toxin incorporation into lipid monolayer (Colacicco and Buckelew 1971) and reduce the amplitude of the early receptor potential (ERP) in the frog eye (Glubo and Petrosini 1973). These effects of low urea concentrations have been interpreted to reflect swelling of peptide chains, due to effects on hydrophobic interactions and/or hydrogen bonds, within the membrane. In addition 200 mM urea has been shown to prevent contact-inhibition of growth of fibroblasts, which is interpreted as an effect on some cell-surface constituent, probably a protein (Weston and Hendricks 1972).

Very little work has been done on the effects of urea on the membrane properties of ex able cells It was found (Bárány Bárány and Trautwein 1960) that urea in a concentration 1 0 M does not affect the action potential in frog muscle, while Tasaki, Barry and Carnay 971) showed that urea affects the change in fluorescence which accompanies the action tential in the crab nerve. However no systematic study has been done on the effects of ea on the resting membrane potential and the action potential. The present study con- rns the effect of urea on passive properties, such as the resting membrane potential, input sistance and specific membrane resistance, and on the action potential of the frog muscle ll membrane. In order to study the effects on passive membrane properties, analysis was so carried out of the effect of urea on the electrolyte composition of the muscle fibres.

Methods

xperimental procedure

artorius muscles from *Rana temporaria* ere used. The muscle was carefully dissected and mounted in a perspex chamber (about 5 ml) ith its deep surface upward and stretched to about 110–120 of its resting ngth. The preparation was maintained at room temperature (about 23°C)

Two microelectrodes ere inserted into the f bres, one electrode for injecting current and the other for cording potential changes. The electrodes used to measure potential changes were glass capillary micro-lectrodes filled ith 3 M KCl, resistance 5–15 MΩ and tip potential less than 5 mV The microelectrodes sed to inject current were filled ith 2 M potassium citrate and had resistance of about 10 MΩ. The cording electrode was introduced into the fibre and the membrane potential recorded, and then the cur ent electrode was introduced and rectangular current pulses (duration 300 msec) were passed. The resulting otential changes ere recorded ith the current electrode inserted successively at three locations at dis- tances varying from 200 to 1 200 μm from the recording electrode The distances were measured through he microscope ith an accuracy of about ± 10 μm. For every location three different values of the current and the potential change were recorded At the end of the experiment an action potential was elicited by depolarizing pulse As result of the contraction of the muscle the electrode was usually dislodged About 0–60 before the pulse was delivered the membrane was polarized locally at the site of measurement to 90 mV

Membrane potential changes ere displayed on an oscilloscope through high input impedance ampli- fier In addition the resting membrane potential was monitored on digital voltmeter The currents were displayed on the oscilloscope as the voltage drop across 200 kΩ feed-back resistor over an operational amplifier used to hold the bath at ground potential, g the bath was connected to the virtual ground of the amplifier coupling. The rate of rise of the action potential was obtained by differentiating the action potential through Miller differentiator and was displayed together ith the action potential.

Acta physiol. scand 1977 *100* 56–68

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Effect of Urea on Some Electrophysiological Properties of the Frog Muscle Cell Membrane

By

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Received 11 October 1976

Abstract

BOËTHIUS, J and B RYDQVIST *Effect of urea on some electrophysiological properties of t frog muscle cell membrane* Acta physiol scand 1977 *100* 56–68

The effect of urea on the electrophysiological properties of the frog muscle cell membrane was stud with intracellular microelectrodes, t gether with an analysis of the electrolyte composition of the mus The d fferent parameters were measured and evaluated after soaking the muscle for 60 min at urea c centrations up to 2.25 M The resting membrane potential was markedly decreased bove 1.50 M and t to bout −50mV at 2.25 M The specific membrane resistance (R_m) was almost naffected t concent tions of 0 75 M and 1.50 M but was reduced after 60 min in 2 25 M to very low values indicating a lea membrane. The maximum rate of rise of the act on potential ($\dot{V}_A$) was unaffected up to 0.50 M but w reduced to about 50% of control value at 0 75 M Between 0.75 M and 1.25 M it was constant and higher concentratio s reduced to almost zero The reduction f $\dot{V}_A$ at the lower concentrations was n accompanied by changes in neither the resting membrane potential nor R_m. It is proposed that urea p turbs protein systems concerned with the generation of the muscle action potential without affecting t passive electrical properties of the muscle membrane. The electrolyte analysis revealed an increase in int cellular N and K concentrations, mainly due to loss of i tracellula water

The main components of excitable membrane are proteins and lipids which presumably some extent are held together by the same general types of bonds which determine t tertiary and quarternary structure of proteins Very little is known of the molecular bas of the various functional membrane properties but according to the fluid mosaic mod protein rich regions are responsible for such functional properties of the membrane permeability transport mechanisms and receptor functions, while more purely lipid regio are responsible for the integrity and cohesiveness of the membrane structure (Singer an Nicolson 1972) In view of this, urea, a well known agent capable of unfolding proteins thereby affecting both quarternary and tertiary structures—can be assumed to affect sens tive membrane functions, for example excitability in nerve and muscle.

A considerable amount of work has been carried out with urea on different biologic systems thus early work (Tanford, 1964 1968 1970 Nozaki and Tanford 1963) has show that small globular proteins can undergo transitions between the native and the denaturate

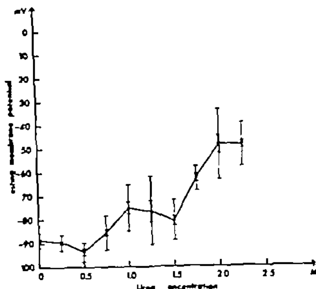


Fig 1 Resting membrane potential at various urea concentrations after 60 min exposure Thin bars indicate ±S D and thick bars S E

iodium, potassium and chloride were determined with neutron activation analysis (Bergström 1962) l potassium and magnesium determinations were made ith atomic absorption flame photometry rgström, Hultman and Solbert 1973) The extracellular and intracellular ater content in the muscle s estimated according to Graham, Lamb and Linton (1967) This method is based on the assumption t the chloride ions are distributed passively over the cell membrane according to the Nernst equation. hen the resting membrane potential is known (and since external chloride concentration is also known) possible to calculate external and internal ater content (see also Bergström, Bohthius ad Hultman 70)

Results

embr electrical properties

ig. 1 shows the effect of the various urea solutions on the resting membrane potential after) min exposure. Between 0.25 M and 0.75 M urea there is no major change in the potential The potential values with concentrations between 1.00 M and 1.50 M are somewhat ower (ca. 10 mV) than at lower concentrations. For concentrations above 1.50 M there is a narked decrease in the potential. Between 2.00 and 2.25 M the potential seems to level off A value of about 50 mV It thus appears that the decrease in the magnitude of the membrane potential with increasing urea concentration may approximate a step-function with one possible step at 1.5–1 75 M.

The effects of different urea solutions on the input resistance (R_{in}) and the specific membrane resistance (R_m) as a function of time are given in Table I for three concentrations 0.75 1.50 and 2.25 M. This table includes values up to 75 min except for 1.50 M where values up to 105 min are included. Table I also includes values for the length constant (λ) and the calculated f bre diameter (d) For 0.75 M there is no marked decrease n either R_{in} or R_m (for R_m see also Fig. 2) up to 60 min. At 75 min there is a small decrease in R_m. The R_{in} alues at 1.50 M are quite similar to those at 0.75 M except the increase at 30 min. At a concentration of 2.25 M the R_{in} increase to over 400 kΩ after 30 min and then steadily

Membrane constants were obtained by rectangula pulse analysis for the case of an infinite cable (Hodgkin and Rushton 1946, Katz 1948 Fatt and Katz 1951 Hodgkin and Nakajima 1972).

The space constant λ and input resistance $R_{in} = \frac{1}{2}\sqrt{r_m r_i}$ were obtained from the distribution of potentials in the steady state using the relation $V(0,\infty) = \frac{1}{2} I(x) \sqrt{r_m r_i} \exp(-x/\lambda)$ where

$\lambda = \sqrt{r_m/r_i}$

x is the intraelectrode distance

r_m is the resistance times unit length of the surface membrane of the fibre in Ω cm

$$\left(r_m = \frac{R_m}{2\pi a}\right)$$

R_m is the specific membrane resistance in Ω cm²

r_i is the resistance per unit length of axis cylinder in Ω cm⁻

$$\left(r_i = \frac{R_i}{\pi a^2}\right)$$

R_i is the internal specific resistance in Ω cm

a is the radius of fibre

The equation was transcribed into the form

$$\log R(x) = -0.4343x/\lambda + \log R_{in}$$

$$\text{where } R(x) = \frac{V(0\ \infty)}{I(x)}$$

For each of the three x values the mean of three $\log R(x)$-values was computed and used to get a regression line, which should be a straight line in a log diagram. The intercept with the $\log R(x)$ axis ($x = 0$) is $\log R_{in}$ and the slope gives λ. The regression line was tested f linearity with F-test at the 1% significance level nd experiments which did not fulfill this criterion were excluded. From R_{in} and λ, R_m and a was computed assuming $R_i = 200\ \Omega$ cm (F tt 1964) All parameters presented in figures and tables as mean standard deviation for t me T are computed o basis of individual values between $T-7.5$ and $T+7.5$ except for T 15 min In this case the corresponding measurements were made for values between 10 and 20 min

Solutions

The ormal Ringer solution contained N Cl 115 mM KCL 2.5 mM $CaCl_2$ 1.8 mM Na_2HPO_4 2.15 mM NaH_2PO_4 0.85 mM (Adrian 1956). A stock solution of rea (nalytical grade) in Ringer solution was made up (3 M) just before the experiment and diluted with normal Ri ger t the actual concentration and This was done order to prevent formation of cya t which takes place if urea solutions become old (Stark, Stein nd Moore 1960). T reduce the osmotic mpact on the membrane, the concentrated urea Ringer solutions were pplied in steps. Up t concentratio of 0.75 M urea solutions were pplied directly For concentrations between 0.75 M and 1.50 M the solutions were applied i tw steps, first 0.75 M and after 10 mi the final concentration to be used. For concentrations between 1.50 M and 2.25 M, the final step took place after 20 mi i 1.5 M solution The time of onset was the time when the first urea solution was applied. The effect of the various urea concentrations was compared after 60 min.

The conductances of the urea-Ringer solutions were measured with a Kematron Tetramatic Mho-meter A sl ght linear increase i the spec fic resist vity (Ω cm) w th increasi g concentration wa noted the values were 84 Ω cm for normal Ringer solution and 94 f 2.25 M urea solution.

Electrol t compositio f muscle fibres

Frog *sartorius* muscles were dissected free and care was t ken to remove ll connect tissue, especially at the pelvic ends. 5 muscles were prepared f each concentration f urea R nger solution 4 different solutions were used normal Ringer sol tion, 0.75 M 1.50 M d 2.25 M urea. T reduce the osmotic impact of high concentrations of rea the procedure described bove was applied

The muscles were first placed the urea-Ringer solution for 60 min. The muscles were then removed from the solution, carefully blotted gainst smooth surface nd immediately weighed on M ttler H 1 balance. N correction was made for water evaporation from the preparation The muscles were then placed in desiccator d dried at 70°C for 48 h. After this the muscles were weighted nd placed i dry plastic

TABLE II. Muscle electrolyte composition for muscles soaked for 60 min in different urea solutions. Figures are mean ± S.D.

	Normal Ringer	0.75 M	1.50 M	2.25 M
H_2O ml/100 g FFS*	367 ± 13 (5)	231 ± 9 (5)	214 ± 14 (5)	191 ± 9 (5)
Na meq/100 g FFS	14.0 ± 1.9 (4)	7.8 ± 1.8 (5)	11.7 ± 1.3 (5)	14.5 ± 1.0 (5)
K meq/100 g FFS	41.1 ± 1.8 (4)	38.0 ± 1.4 (5)	29.3 ± 4.4 (5)	20.3 ± 2.1 (5)
Mg meq/100 g FFS	9.6 ± 0.2 (4)	9.1 ± 0.2 (5)	8.3 ± 0.4 (5)	7.7 ± 0.2 (5)
Cl meq/100 g FFS	12.0 ± 2.1 (4)	5.9 ± 1.4 (5)	9.5 ± 1.6 (5)	11.0 ± 1.0 (5)

FFS fat free solid.

It is interesting to note that after 30 min there seems to be no major change in fibre diameter indicating that the volume of the intracellular water is the same as in Ringer solutions. This is not in accordance with the values for intracellular water seen in Table III which shows a clear decrease for all concentrations presented. The reason for this discrepancy is not clear but a change in internal resistivity (R_i) might explain the results obtained (see Discussion).

Muscle cell electrolytes

Table II shows the contents of water, sodium, potassium chloride and magnesium of muscles soaked in urea for 60 min. As expected, exposure to increasing urea concentrations causes water and potassium to leave the muscle, as indicated by the decrease of the concentrations of these substances at increasing urea concentrations. Sodium and chloride decrease at low urea concentrations, they then tend to increase again in more concentrated urea solutions, when the muscle cell membrane becomes more permeable to these ions. This pattern presumably corresponds to a first phase when the extracellular fluid is osmotically extracted from the muscle, and a second phase when sodium and chloride enter the muscle fibres. The changes in intracellular magnesium indicate a slow leakage of this ion out of the muscle fibre with increasing concentrations.

Table III shows the calculated values for intracellular ions and extra- and intracellular water at the different urea concentrations. As expected, both intra- and extracellular water decrease as the urea concentration is increased.

TABLE III. Derived values for water and electrolytes in muscle soaked for 60 min in different urea solutions. Figures are mean ± S.D.

	Normal Ringer	0.75 M	1.50 M	2.25 M
H_2O_e ml/100 g FFS	84 ± 16 (4)	38 ± 11 (5)	47 ± 13 (5)	65 ± 8 (5)
H_2O_i ml/100 g FFS	284 ± 3 (4)	195 ± 8 (5)	147 ± 19 (5)	125 ± 7 (5)
Na_i meq/100 g FFS	3.9 ± 0.4 (4)	3.3 ± 0.6 (5)	3.6 ± 0.5 (5)	6.6 ± 0.4 (5)
Na_i meq/l	13.8 ± 1.3 (4)	16.9 ± 3.2 (5)	25.0 ± 3.1 (5)	52.7 ± 1.4 (5)
K_i meq/l	144.4 ± 6.6 (4)	194.2 ± 13.0 (5)	200 ± 24 (5)	163 ± 23 (5)
$(Na_i + K_i)/(H_2O_i)$, meq/l*	150 ± 6 (4)	196 ± 9 (5)	192 ± 15 (5)	183 ± 14 (5)
RMP* mV	89	85	80	−49
P_{Na}/P_K	0 016	0 029	0 453	0.206

* Calculated on basis of electrolyte and water content in Table II

TABLE I Input resistance (R_{in}) and length constant (λ) as experimentally measured according to Meth specific membrane resistance (R_m) and diameter of muscle fibres, as calculated according Methods, exposed to 3 urea solutions Values are mean ± S.D Diameters of fibres are comp. assuming internal resistivity (R_i) 200 Ω cm (see text).

T (min)	n	R_{inl} (M Ω)	λ (mm)	R_m (Ω cm²)	d (μm)
			0 75 M		
0	8	0 21±0.08	2.22±0 70	3 630± 250	118±[illegible]
15	9	0.[illegible]1±0.04	2.10±0.83	3 [illegible]50±2 120	113±24
30	11	0.31±0.25	2.22±1.07	3 830±2 530	110±42
45	5	0.33±0.[illegible]1	2.07±0.82	3 530±1 610	103±43
60	12	0.20±0.09	2.34±1.37	3 420±2 310	131±66
75	14	0.[illegible]2±0.10	1 83±0.88	2 570±1 870	109±36
			1.50 M		
0	29	0.24±0.10	[illegible]1±0.92	3 540±1 930	116±42
15	0				
30	10	0.35±0 18	[illegible]12±0.50	4 100±2 040	95±[illegible]3
45	5	0.25±0.11	[illegible]49±0.9	4 140±1 670	1[illegible]0±44
60	10	0.26±0 12	2.46±0.95	4 320±2 220	117±40
75	5	0.21±0.06	1 71±0.44	330± 900	103±16
90	7	0.22±0 05	2.10±0 57	3 [illegible]30±1 240	111±[illegible]3
105	12	0 17±0.05	2.22±0 88	3 310± 210	126±[illegible]
			2.[illegible]5 M		
0	15	0.26±0.16	2.51±0.62	4 340±1 670	125±40
15	0				
30	8	0 42±0.25	1 93±0 58	3 730±1 790	85±31
45	4	0.29±0.35	[illegible]56±1 68	3 200±1 870	161±117
60	12	0.19±0.17	1 15±0 60	1 130± 930	109±51
75	5	0.08±0.04	1 03±0.43	600±260	146±66

declines to very low values. It seems reasonable to assume that an osmotically-indu shrinking of the muscle fibre is an important factor underlying the increase in R_m at 30 min in both [illegible].25 M and 1.50 M As can be seen the calculated fibre diameter at 30 mi lower than the control values for the two concentrations.

At high urea concentration (2.25 M) R_m is seriously affected At 45 min R_m is marke reduced, and it reaches very low values at 60 and 75 min

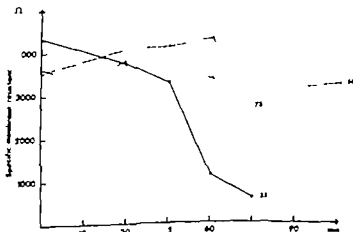


Fig. 2. Specific membrane re[illegible] ance (R_m) as functio of time three urea solutions. See T[illegible] f S D and number of f bres.

Fig. 4. Action potentials elicited by ramp stimuli of fibres soaked in different urea solutions for 60 min. A, control, B 0.75 M C 1.50 M, D 1.75 M. Upper trace displays the differentiated signal.

The effects of various concentrations of urea on the heights and thresholds of the action potentials are shown in Fig. 5. The height of the potential remains unchanged up to 1.25 M. At 1.75 M the overshoot has disappeared, but using strong stimuli it is usually possible to reach the zero-level. The threshold remains constant up to 0.50 M with higher concentrations there is a gradual increase in threshold.

For a membrane action potential with zero longitudinal current the membrane current I_m is related to the rate of rise of the action potential according to the equation

$$I_m = C_m \frac{dV}{dt}$$

where $C_{[illegible]}$ is the membrane capacitance (Hodgkin and Katz 1949). This relation may also be valid for the muscle fibre and the same equation has therefore been used for estimating the membrane current. The maximum rate of rise of the action potential is believed to reflect the maximal sodium conductance (cf. Hibbard, Llinás and Quastel 1969; Theslef 1971). To make sure that the membrane capacitance was not changed during urea treatment this parameter was evaluated using [illegible] $= R_m C_m$, where [illegible] was taken as the time for an electrotonic potential to reach 83% of its maximum value. These values showed that no significant ([illegible] significant level) change takes place after 60 min at the three concentrations 0.75, 1.50 and 2.25 M (Table IV). The rate of rise is a sensitive parameter and the plot of the maximum rate of rise versus urea concentrations (Fig. 6) reveals several points of interest. The rate of rise remains at the control level up to 0.50 M. At 0.75 M it is reduced to about 50% of the control value. After this steplike fall, there is a plateau in the curve from 0.75 M to 1.25 M. From 1.25 M there is a steady decline in the rate of rise, which almost reaches zero at 2.00 M. The curve thus approximates a

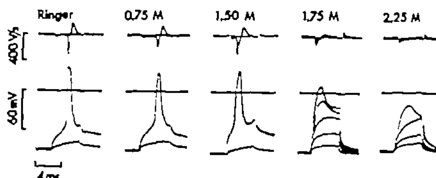


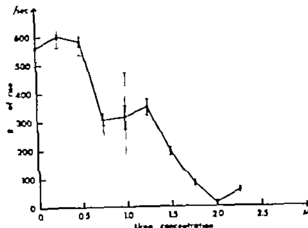
Fig. 3 Action potentials in muscle fibres soaked in urea solutions for 60 min. Upper trace shows the differentiated signal.

When the amount of intracellular sodium is related to the dry weight there is a change up to 1.5 M urea. At 2.25 M the amount of intracellular sodium has doubled, thus indicating an increase in the sodium permeability of the muscle cell membrane. In the case of intracellular potassium concentration there is an increase from the control value of 144 mM to 200 mM for the muscle treated with 1.50 M urea, presumably due to shrinking of the fibre. Between 1.50 M and 2.25 M urea there is a decrease in intracellular potassium of about the same magnitude as the corresponding increase in intracellular sodium.

The concentration values for sodium and potassium in Table III were used to calculate the relative sodium permeability (P_{Na}/P_K) according to the Goldman equation (Goldman 1943, Hodgkin and Katz 1949) The values were 0.016 in Ringer 0.029 in 0.75 M urea, 0.033 in 1.50 M urea and 0.206 in 2.25 M urea. The changes in electrolyte concentration *per se* are thus not sufficient to explain the observed decrease in the resting membrane potential. It therefore appears that the changes may be a consequence of changes in the permeability properties of the membrane

Active electrical properties

Fig. 3 shows a series of muscle action potentials recorded after 60 min in various urea concentrations. The action potentials of the muscle fibres treated with 0 75 M and 1.50 M urea show the same general configuration as the control However although the maximum amplitude is approximately the same, the maximum rate of rise is quite clearly less, and the impulse duration longer than that of the control The potentials which were recorded from muscles immersed in 1 75 M and 2.25 M urea respectively are on the other hand abortive and seem to have lost their "all-or-nothing" characteristics. The height has diminished and the duration greatly increased and the rate of rise has decreased to very low values. In view of these observations that the membrane loses its ability to produce all-or nothing spikes at concentrations above 1 75 M it appeared of interest to investigate the effect of ramp stimuli (*cf* Frankenhaeuser and Vallbo 1965 Vallbo 1964) Such recordings are shown in Fig. 4 At a urea concentration of 0 75 M (4 B) there is a definite decrease in the rate of rise and the height of the action potential at low rates of rise of the ramp At a concentration of 1 75 M (4 D) it is only possible to elicit small graded "hump-like" potentials which bear ... obtained at low concentrations.



ig. 6. Maximum rate of rise of action potential of muscle fibres soaked in different urea solutions for 60 min. Thin bars indicate ± S.D. and thick bars ± S.E.

In muscle fibres treated with urea the resting membrane potential remains near normal up to 1.50 M (Fig. 1). Within this range the input resistance (R_{in}) and specific membrane resistance (R_m) also remain near their control values (*cf.* Table I and Fig. 2). At higher urea concentrations there is a significant fall in resting membrane potential and also in R_{in} and R_m. The changes in electrolyte composition at increasing urea concentrations indicate an increasing relative sodium conductance which is also borne out by the calculated P_K/P values.

It thus appears that urea solutions in concentrations up to 1.50 M have little effect on the passive electrical properties of the membrane. However there is a small but definite increase in the relative sodium permeability and a close inspection of Fig. 1 reveals small decrease of about 10 mV in the mean membrane potential of about 0.75 M urea, which may correspond to the early increase in sodium permeability.

At urea concentrations higher than 1.50 M there is a drastic decrease in the membrane potential (see above) which seems to correspond to a marked decrease in the potassium-selectivity of the membrane. This phenomenon appears to be connected to a general increase in membrane conductance affecting both sodium and potassium channels. This general increase in conductance seems to be characteristic for high urea concentrations but is absent in concentrations below 1.50 M.

The computations of specific membrane resistance (R_m) assume a constant internal resistivity (R_i). However it is possible that the R_i of the fibre changes. Comparing calculated fibre diameter (Table I) and water loss from the fibre (Table III) it is probably clear that R must change to some extent. If it is taken into consideration the increase in K and Na after 60 min in urea solution, which should give a lower value for R_i it is reasonable to assume that the computed values for the fibre diameter are too high for the urea concentrations presented. However this can only be stated for the 60 min values since no electrolyte concentrations have been measured for other times.

If it is assumed that R_i is inversely proportional to the sum of the K_i and Na concentrations the following calculations for 2.25 M at 60 min should be valid. K and Na are increased to 15 meq/l from 157 meq/l (control) which equals a decrease in R from 200 to

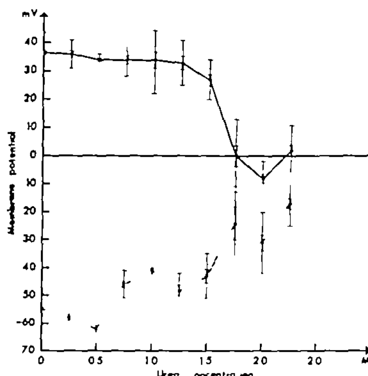


Fig. 5 Amplitude (solid line) and threshold (dotted line) of action potentials of muscle fibres soaked in different urea solutions for 60 min. Thin bars indicate ±SD and thick bars indicate ±S E.

double" step function with sharp falls at 0.50 M and 1.25 M In view of the denaturating properties of urea, the steps in the curve might correspond to molecular transitions within the membrane.

Discussion

Urea has long been an important tool in protein chemistry It is used mainly to disrupt the tertiary and quarternary structure of proteins The mechanisms are incompletely understood It has been proposed that urea 1) breaks the ordering of water 2) breaks hydrogen bonds, 3) disrupts hydrophobic interactions, 4) interacts directly with the protein molecule However despite the considerable uncertainties on those points, the close study of the effect of urea on various proteins has yielded valuable data on protein structure and function (Snape *et al* 1974) Urea is also one of the fastest permeating nonionic solutes with a permeability constant of 23 10^{-6} cm/s (Naccachie and Shaafi 1973) and a halftime of penetration through the cell membrane of 15 min (Bozler 1959). Also urea space for *sartorius* muscle cells is slightly higher than that of water (Bozler 1959) This makes a mechanical effect of the hyperosmolarity on the cell membrane less probable

TABLE IV Membrane capacitance (μF) for muscle fibres immersed urea solutions for 60 min. Figures are mean ±S D

T (min)	0 75 M	1 50 M	2 25 M
0	10.8±4 4 (8)	9.5±3.8 (15)	9.0±6.4 (10)
60	10.7±3 9 (14)	8.3±5 0 (14)	11 6±7.0 (16)

Control values and 60 min values equal for all concentrations on 1% significance level

In view of the differences between the effect of urea on the resting membrane potential d R_m (Fig. 1 and 2) and the effect of urea on $\dot{V}$ (Fig. 6)—$\dot{V}$ being reduced to about 50% control values at 0.75 M while at the same concentration resting membrane potential d R_m is very little changed—it seems probable that urea does not affect the passive permeability properties presumed to be related to the lipid phase while urea has a specific effect n the mechanism underlying the action potential. This mechanism could well be a protein stem.

At higher concentrations the integrity of the membrane seems to be affected, leading to duction of resting membrane potential and R_m.

he authors are greatly indebted to Dr J. Bergström who performed the muscle electrolyte analysis.
This work has been supported by grants from Karolinska Institutet and Svenska Sällskapet för Medicinsk orskning.

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146 Ωcm. According to the algorithm for computing the fibre diameter this yields a fibre diameter of 93 μm instead of 109 μm for 60 min. The diameter is thus reduced from 125 μm (control) to 93 μm and the associated cell volume is reduced 45 %, which should be compared with the water loss from 284 ml/100 g FFS to 125 ml/100 g FFS (Table IV), a decrease of 56 %, i.e. the two methods yield a decrease in volume of corresponding magnitude. The decrease in R_i calculated above, would also lead to a reduction of the calculated values for R_m with about 15 %.

One typical effect of urea on the action potential is to increase accommodation, making the potential similar to a graded response at higher urea concentrations. This is especially evident in the effect of urea on the rate of rise of the action potential. It is well known that a depolarization of the muscle membrane can cause similar changes (Knutsson 1966, Knutsson and Skoglund 1969). However, in the present experiments the increased accommodation occurred when the membrane potential was only insignificantly reduced. Furthermore, at these concentrations the increase in P_{Na}/P_K is so small, that, with R_m unchanged, any major shunting effect seems unlikely. In view of this it seems reasonable to conclude that the increase in accommodation is caused by an effect of urea on the action potential generating mechanism.

At increasing urea concentrations the rate of rise of the action potential decreases in two steps. The first steep decline in the curve starts at 0.50 M urea and the second one at 1.25 M. Between 0.75 and 1.25 M there is a plateau. Curves of this general shape may indicate transitional phenomena. A crystallographic study of the interaction of urea with lysozyme (Snape et al. 1974) shows that the main urea induced conformational changes in this protein appear already at a urea concentration of 2.00 M, viz. the range used in the present study. It is tempting to speculate that the "steps" in the rate of rise curve represent urea-induced conformational transitions in some protein or proteins intimately concerned with the generation of action potentials.

An alternative explanation for the decrease of the maximum rate of rise (V_A) of the action potential would be that urea changes the activity of some ions, in particular Na and Ca. Decreased Na and increased Ca activities reduce V_A.

However, urea is known to affect Na activity very little (Bower and Robinson 1967), which is consistent with our finding that conductivity is very little affected, whereas Ca activity is reduced, as reported by Finlayson, Roth and Dubois (1972). Their results show that Ca activity in $CaCl_2$ solutions is reduced to about 50 % in a 2.25 M urea solution. This decrease in activity will be reduced when the ionic strength of the solution is increased. Consequently it seems highly unlikely that changes in ionic activities are responsible for the decrease in V_A.

The effects on resting membrane potential (Fig. 1) suggested at least one clear "step". The step in this curve is separate from the steps in the curve depicting the maximum rate of rise (Fig. 6). The passive permeability properties of the membrane are a less complex function of changing urea concentration than the action potential, and may be more dependent on the lipid than on the protein part of the membrane. It is therefore possible to extend the speculation and suggest that the "step" in the curve showing the resting membrane potentials reflect urea-induced transition in the lipid phase of the membrane.

Acta physiol. scand. 1977. 100. 69–83

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Mechanical, Electrical, and Biochemical Effects of Hypoxia and Substrate Removal on Spontaneously Active Vascular Smooth Muscle

By

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Received 21 October 1976

Abstract

HELLSTRAND, P., B. JOHANSSON and K. NORBERG. *Mechanical, electrical and biochemical effects of hypoxia and substrate removal on spontaneously active vascular smooth muscle.* Acta physiol. scand. 1977. *100*. 69–83.

Effects of hypoxia and glucose-free solution on the isolated rat portal vein were studied. Decrease of extracellular P_{O_2} below 50 mm Hg caused graded inhibition of spontaneous mechanical activity; below 7 mm Hg, inhibition was complete in most preparations. Contracture force of depolarized portal vein was less sensitive to decreases in P_{O_2}. Responses to noradrenaline at all concentrations were markedly depressed at extreme hypoxia. Sucrose-gap experiments showed that hypoxia reduced the spontaneous electrical spike discharge. Mean tissue contents of PCr, ATP and glycogen (expressed as glucose) were 3.02, 2.47 and 5.07 µmol/g cell ... in spontaneously active control muscles and 1.07, 1.65 and 1.83 after 20 min anoxia. Physiological variations in P_{O_2} may influence myogenic activity of vascular smooth muscle largely through an action at the membrane level and this mechanism may participate in local blood flow control. Calculations indicated that the graded response to hypoxia in the present in vitro experiments was not due to diffusion limitation. Spontaneous mechanical activity was relatively well maintained even after prolonged exposure to glucose-free solution, whereas the responses to K⁺ and noradrenaline were markedly suppressed. Electrophysiological recordings during spontaneous activity indicated desynchronization and impaired conduction. PCr and ATP were maintained at control levels and glycogen reduced by 50 per cent after 2 h in glucose-free medium. Indications of the use of amino acids (glutamate) as substrate under these conditions were obtained.

Blood flow is adjusted to the metabolic activity of different tissues mainly through local control mechanisms. Physicochemical factors, related to metabolism, which produce functional hyperemia through changes in tone of the resistance and precapillary sphincter vessels have been the subject of much research and debate over the last century (for ref. see Mellander and Johansson 1968). It appears that functional hyperemia is brought about not by one single factor but by cooperation of several factors which vary in their relative importance in different tissues.

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situation in the present study. Vigorous bubbling was used throughout the experiments to obtain rapid gas equilibration. Mixing of the solution was further improved by a motor-driven stirrer. A parafilm cover on the bath, with holes for the connections to the transducers, minimized the contact between bath fluid and ambient air. The oxygen tension in the bath was recorded by an oxygen electrode (L. Eschweiler & Co, Kiel, Germany). After the preparation had stabilized in the standard medium as indicated by the pattern of spontaneous activity it was exposed to graded degrees of hypoxia obtained by mixing the control gas with 4 % CO_2 in N_2 through a valve system.

In other expts the muscle was transferred to glucose-free solution, i.e. standard Krebs medium in which glucose had been replaced by isosmotic amounts of NaCl. Bubbling with the control gas containing 16 % O_2 was maintained throughout these expts.

The effects of hypoxia or glucose removal on the spontaneous contractile activity were quantified by electronic integration of the active isometric force. A Grass polygraph was used for recording. In separate sets of experiments the effects of hypoxia or glucose elimination on the potassium contractures of the portal vein were examined. Contractures were elicited by solutions in which equimolar amounts of KCl replaced all NaCl of the standard or of the glucose-free medium, respectively. Also the effects of hypoxia and glucose depletion on responsiveness to noradrenaline were studied in separate experiments by running cumulative concentration-response curves with this agent (see further below).

Care was taken not to expose the muscle to air during the change of solution in the hypoxia expts.

Sucrose-gap experiments

Simultaneous recording of electrical and mechanical activity were carried out by the sucrose gap method (see Axelsson *et al.* 1967). In these expts the effects of glucose-free Krebs or of control medium bubbled with 4 % CO_2 in N_2 were examined. Oxygen tension could not be recorded near the preparation and the degree of hypoxia is therefore somewhat uncertain as some uptake of O_2 from the ambient air may have occurred between the pre-aeration chamber and the sucrose-gap apparatus. A comparison of the mechanical responses in these and the above experiments indicated, however, that the preparations were exposed to P_{O_2} < 15 mmHg. Isometric force was recorded and integrated as above. The output from the sucrose-gap electrodes was amplified and registered on the Grass polygraph together with the signals from an electronic spike counter which measured the number of action potentials from the appropriately AC filtered electrical activity (see Johansson and Mellander 1975).

Biochemical experiments

Approximately 15 mm long sections of the portal mesenteric vein were dissected from rats which had been kept fasting overnight. The vessels were cut open longitudinally and mounted at their in situ lengths on small stainless steel holders. The preparations were accommodated in standard Krebs bubbled with 96 % O_2 and 4 % CO_2 and were then preincubated for 1 h in control medium equilibrated with the 16 % O_2 gas mixture as described above. They were then transferred to glucose-free Krebs, to standard Krebs equilibrated with 4 % CO_2 in N_2, or to an incubation bath with control medium. These incubations lasted for 2 h in the expts designed to examine the effects of glucose removal and for 20 min in the study of anoxia. The incubation media contained ^{14}C-sucrose (Amersham Radiochemical Centre, 394 mCi/mmol) at an approximate concentration of 0.5 μM. This tracer was used to measure the extracellular fluid including the amount of bath fluid adhering to the preparation. It has been shown that ^{14}C-sucrose distributes itself in the extracellular fluid space of rat portal vein during the first 15 min of incubation and that little change in the distribution volume occurs thereafter (Arvill, Johansson and Jonsson 1969). At the end of the incubation periods the muscles, still on the metal holders, were rapidly (about 1 s) thrown into liquid N_2 for later analysis. In view of the small mass of tissue it was necessary to pool 10 portal veins for each measurement. One control and one test group (anoxic or glucose-free) were prepared and incubated on each experimental day. The preparations were stored at −80 °C. The veins were cut away from the metal holders in a glove box at −22 °C. The samples were weighed and extracted at this temperature with HCl-methanol. The tubes were brought to 0 °C and the extraction completed. The extracts were analyzed for organic phosphates, glycogen, glycolytic intermediates and amino acids by enzymatic fluorometric techniques (Lowry and Passonneau 1972, Folbergrová *et al.* 1972 a, b, 1974 a, b). The radioactivity from the ^{14}C-sucrose was counted in samples of the muscle extracts and of the respective bath media. The cellular wet weight of the portal veins could then be calculated and concentrations expressed in µmol/g cell.

The local oxygen tension has been considered an important factor e g in the produc of functional hyperemia and autoregulation in skeletal muscle (see e.g Guyton et al Skinner and Powell 1967) According to this view increased metabolism in the tissue reduces the local P_{O_2} to which the vascular smooth muscle of the microcirculation is posed this change in P_{O_2} would then inhibit vascular tone directly and not via increa production of vasodilator metabolites by the tissue cells. Implicit in this argument is the that the contractile activity of the vascular smooth muscle is critically dependent on continuous supply of oxygen and, further that the P_{O_2} within the vessel walls of lary microcirculation really drops to levels where this supply is jeopardized Attempts h been made to elucidate the former aspect by direct expts. on isolated vessels in vitro. It been shown that the spontaneous tone of isolated perfused small arteries from dog skel muscle is dependent on the blood P_{O_2} (Carrier Walker and Guyton 1964). Further work the contractile and metabolic effects of hypoxia in vascular smooth muscle has been out mainly on preparations of large arteries stimulated by adrenergic substances (Detar Bohr 1968 Namm and Zucker 1973 Pittman and Duling 1973) Since local control of bl flow mainly results from variations in the myogenic component of tone in the resista vessels (Mellander and Johansson 1968), it appears more relevant to examine the oxy dependence of vascular smooth muscle preparations which in contrast to large arter possess myogenic spontaneous activity The smooth muscle of portal-mesenteric veins several laboratory animals shows characteristic myogenic activity resembling microvasc vasomotion (cf Wiedeman 1966) It is worth noticing that these splanchnic veins are uni in being both pre och postcapillary

The purpose of the present study was to investigate the possibilities for metabolic cont in myogenically active vascular smooth muscle by examining the mechanical, electrical metabolic responses of the rat portal vein to hypoxia and to removal of the exogen substrate.

Some of the results presented here have been reported in preliminary form (Hellstr 1975)

Methods

Portal veins were dissected from male rat of the Sprague Dawley strain with body weights between and 400 g The vessel was cut open lo gitudinally and the blood was removed from the l men

Recording f mechanical actu ty

Isometric contractile activity of the longitudinal muscle layer was measured on 5 t 7 mm long preparati of portal veins which were mo ted in 50 ml organ baths and connected t Grass FT03 force transduc I rder t standardize diffusion conditions between extracellular med m and t ssue as far as possible ends of the preparation were fixed t small platinum holders so that the sl t essel opened and forme rectangular plat of f irly uniform thickness. The eins were llowed t accommodat un tandard Kr solution under passive force of 5 mN for bout 1 h. The composition of this solution in mM was as f lows. N Cl 122 KCl 4 7 NaHCO$_3$ 15 5 KH PO 1 MgCl$_2$ 1 CaCl$_2$ 2.5 glucose 11.5 All experime were carried out t 37°C. D r g the accommodation period the solution was eq hbrated with mi t of 96% O and 4 CO$_2$ g ing a pH of 7 3–7 4 This gas wa then replaced by a mi ture of 16 O$_2$ 4 CO and 80 N$_2$ Standard Krebs solution, equilibrated with this latter gas mixture, represent the cont

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The purpose of the present study was to investigate the possibilities for metabolic control in myogenically active vascular smooth muscle by examining the mechanical, electrical and metabolic responses of the rat portal vein to hypoxia and to removal of the exogenous substrate.

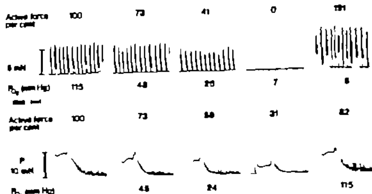
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Methods

Portal veins were dissected from male rats of the Sprague-Dawley strain with body weights between 150 and 400 g. The vessel was cut open longitudinally and the blood was removed from the lumen.

Recording of mechanical activity

Isometric contractile activity of the longitudinal muscle layer was measured on 5 to 7 mm long preparations of portal veins which were mounted in 50 ml organ baths and connected to Grass FT03 force transducers. In order to standardize diffusion conditions between extracellular medium and tissue as far as possible the ends of the preparation were fixed to small platinum holders so that the slit vessel opened and formed a rectangular plate of fairly uniform thickness. The veins were allowed to accommodate in standard Krebs solution under a passive force of 5 mN for about 1 h. The composition of this solution in mM was as follows: NaCl 122, KCl 4.7, $NaHCO_3$ 15.5, KH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 11.5. All experiments were carried out at 37°C. During the accommodation period the solution was equilibrated with a mixture of 96 % O_2 and 4 % CO_2 giving a pH of 7.3–7.4. This gas was then replaced by a mixture of 16 % O_2, 4 % CO_2 and 80 % N_2. Standard Krebs solution, equilibrated with this latter gas mixture, represents the control



g. 2 Spontaneous mechanical activity (upper part) and potassium contractures (lower part) of rat portal in in control medium (P_{O_2} 115 mmHg), during graded levels of hypoxia, and during recovery. F gures active force represent integrated force expressed as percentage of the control activity

hile spike frequency decreased to 23.6 ± 5.9 per cent. The decrease in phasic electrical ctivity during hypoxia appeared in all experiments to be associated with a reduction in the esting membrane potential although this cannot be judged with certainty from sucrose-gap ecordings. A reduced steepness in the depolarizing prepotential preceding each burst of pikes was evident in many recordings (*cf* Fig. 1 A and B).

2 Quantitative aspects of mechanical responses to variations in P_{O_2}

The upper part of Fig. 2 show original recordings of spontaneous contractile activity in a portal vein exposed to graded changes in P_{O_2} of the Krebs solution. Reducing P_{O_2} from the control value at 115 mmHg (standard 16% O gas mixture) to 48 mmHg had little effect on the amplitude of the spontaneous contractions but reduced their duration, so that integrated active force decreased to 73% of control. The further reduction in P_{O_2} to 25 mmHg affected both amplitude and duration of contractions; integrated force therefore fell to 41%. At 7 mmHg mechanical activity was completely inhibited in this preparation. Recovery at the control P_{O_2} was associated with a temporal enhancement of activity to 191% of the initial level as illustrated by the last panel.

In view of the fact that hypoxia appeared to inhibit spontaneous contractile activity largely through an effect on electrical membrane events (section 1 above) it was considered of interest to investigate also the influence of P_{O_2} on the contracture force of depolarized portal veins. In the lower part of Fig. 2 are shown contractures elicited at different P_{O_2}. The active force developed during the second, sustained, phase of the contracture is expressed as a percentage of the control response at 115 mmHg (shown by a dot). Contracture force declined with decreasing P_{O_2} but was not abolished entirely even at P_{O_2} of 1 mmHg. No enhancement of the contracture force appeared in the control period after hypoxia. Notice that the initial, phasic part of the tension response is well maintained even at low P_{O_2}.

Fig. 3 summarizes the quantitative changes in spontaneous mechanical activity produced by variations in P_{O_2} in 5 portal eins. Responses are given as a percentage of the integrated

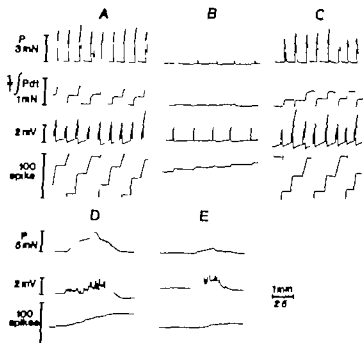


Fig. 1 Sucrose gap recordings of electrical and mechanical activity in isolated rat portal vein. The traces represent isometric active force, integrated active force sampled over 1 min periods, electrical DC activity and output from electronic spike counter. Spontaneous activity in standard medium (A), after 5 min in hypoxic solution (B) and after 15 min recovery (C). Lower part of the figure shows recordings at higher paper speed obtained during control period (D) and during hypoxia (E)

Results

1 *Effects of hypoxia on electrical and mechanical activity*

Fig. 1 exemplifies recordings of electrical and mechanical activity of a portal vein in control solution (A) after 5 min of hypoxia (B), and after 15 min recovery in the control medium (C). The tracing at the top is isometric contractile force, followed by the integral of the active force sampled over 1 min periods the DC recording of electrical activity and the output from the electronic spike counter. One contraction from the control period (D) and one from hypoxia (E) are shown below at higher paper speed.

In the control situation (A) the preparation showed the phasic myogenic contractions associated with bursts of action potentials which are characteristic of the portal vein in normal Krebs solution. The mechanical activity corresponded to a time average of active force of 0 65 mN and the mean spike frequency was 79/min. Hypoxia (P_{O_2} < 15 mmHg) led to a rapid and marked inhibition of spontaneous activity as shown in panel B, so the integrated force after 5 min had decreased to about 0 03 mN. The electrical recording showed a decrease both in frequency of bursts and in number of spikes per burst so that the mean spike frequency became reduced to 4 3/min. The temporal correlation between electrical and mechanical activity was maintained during hypoxia. Return to control led to full recovery of electrical and mechanical activity (C)

In a total of 12 exposures to hypoxia in sucrose-gap expts on 5 portal veins a reduction of integrated contractile force to 14 7 ± 3 8 (mean ± S E.) per cent of the control was found

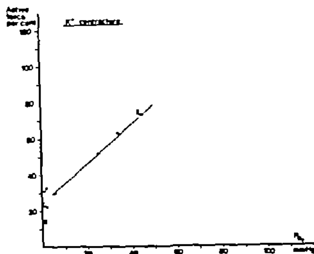


Fig 4 Contracture force elicited by 122 mM K with 5 mM Ca^{2+} as a function of P_{O_2} in 6 portal veins. Results expressed as Fig 3

blocked by propranolol 10 μM in the Krebs solution and neuronal NA uptake was blocked by LU-3-010 0 1 μg/ml (Petersen et al 1966). Three concentration-effect curves were obtained in each expt one during the initial control period, one during hypoxia (P_{O_2} 10 mmHg), and one in the subsequent recovery period. The responses of each individual preparation were expressed as a percentage of its maximal NA response in the initial control run. Fig. 5 summarizes the results of these experiments. It is evident that hypoxia caused a pronounced depression of the entire NA dose-response curve and that this effect was completely reversible A comparison of normalized dose-response curves indicated that hypoxia caused no appreciable shift in ED_{50}

(c) *Effects of glucose-free solution on electrical and mechanical activity*

Removal of the exogenous supply of metabolic substrate had a much less dramatic effect than hypoxia on the spontaneous myogenic activity Fig. 6 exemplifies sucrose-gap record-

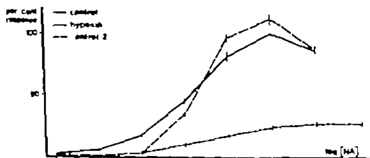


Fig 5 Cumulative concentration-response curves to noradrenaline for 6 portal veins before (control 1), during, and after (control 2) hypoxia (P_{O_2} 10 mmHg) Responses of each muscle expressed in per cent of its maximal integrated force in the initial control run. Vertical bars represent S E. For further details see text.

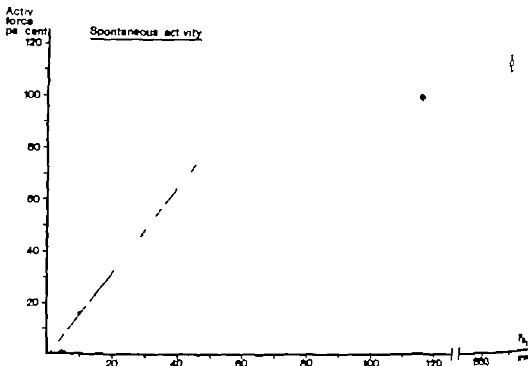
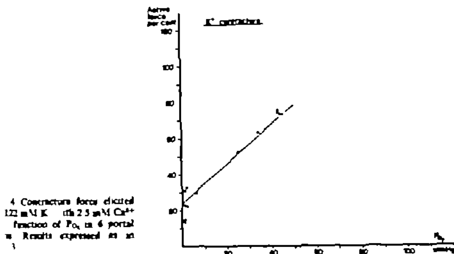


Fig. 3 Integrated active force during spontaneous activity as a function of P_{O_2} in 5 portal veins Data the ind vidual muscle expressed n per cent of its integrated active force at P_{O_2} = 115 mmHg. Broken re sion line summarizes th data for P_{O_2} values < 50 mm Hg. Open circle shows mean ±SE of activity P of 670 mmHg. Mean thickness of muscle sheets 0 11±0.01 (SE) mm.

contractile force in the control period at 115 mmHg before hypoxia. It is seen that sp taneous activity is quite well sustained down to P_{O_2} around 50 mmHg but decreases rap at lower values. High P_{O_2} (670 mmHg) caused little increase in active force above that in control medium. The results of 6 expts on K -contractures are shown in Fig. 4 The ac force was calculated as in Fig. 2. A decrease in the mechanical response with reduced occurred over the entire range of P_{O_2} below 115 mmHg but approximately 25 per cent the response remained even at the extreme degrees of hypoxia. It is difficult to sugge mathematical model which, on physiological grounds, would be expected to represent data shown in Fig. 3 and 4 For a comparison of the results obtained in the two set experiments it seemed reasonable, however to describe the data in the limited P_{O_2} ra < 50 mmHg by straight lines as shown in the diagrams. The regression line for the contr ture data at P_{O_2} < 50 mmHg shows a slope of 11 %/10 mmHg (Fig. 4) compared to the va of 17 %/10 mmHg for spontaneous activity (Fig. 3)

Also the responsiveness of the portal vein to the physiological neurotransmitter n adrenaline (NA), was examined under conditions of marked hypoxia Cumulative c centration-effect curves to NA were obtained by 5-fold increases of NA concentration the bath every 4th min Responses were quantified as the difference between mean act force during the last 3 min at each concentration and the activity in the control per preceding the first dose. In an attempt to make the responses representative of select α-adrenoceptor activation by the bath concentrations of NA the β-adrenoceptors w



4 Contracture force elicited 122 mM K ... with 2.5 mM Ca⁺⁺ ... function of P_{O_2} in 6 portal ... Results expressed as ...

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Effect of glucose-free solution on electrical and mechanical activity

...emoval of the exogenous supply of metabolic substrate had a much less dramatic effect ...han hypoxia on the spontaneous myogenic activity. Fig. 6 exemplifies sucrose-gap record-

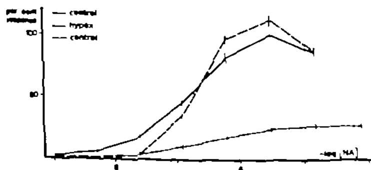


Fig. 5. Cumulative concentration response curves to noradrenaline for 6 portal veins before (control 1), during, and after (control 2) hypoxia (P_{O_2} < 10 mmHg). Responses of each muscle expressed in per cent of its maximal integrated force in the initial control run. Vertical bars represent S.E. For further details see text.

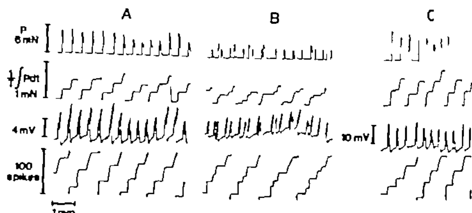


Fig. 6. Sucrose gap recordings of electrical and mechanical activity in isolated rat portal ein. Tracings in upper part of Fig. 1 Spontaneous activity in standard medium (A), in glucose-free solution (B) r during recovery in standard medium (C).

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4 *Quantitative effects of glucose elimination on mechanical activity*

Fig. 7 summarizes the change in spontaneous mechanical activity during 2 h in glucose-fre medium expressed as a percentage of the integrated force during the preceding contr period Contractile activity decreased to about 50 per cent over the 2 h. A partial recover

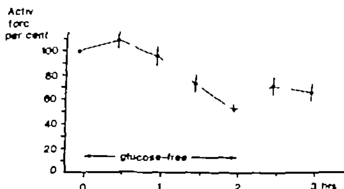


Fig. 7 Cha ge of pontaneous mechanical activ ty during 2 hrs exposure to glucose-free sol tion (9 portal eins) and partial recovery after readministration of normal medium (7 preparations) I tegra force of each muscle in initial control period set to 100 per cent Vertical ba S E.

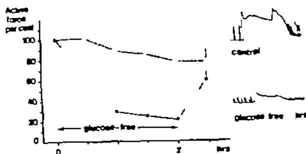


Fig. 8. Effects of glucose-free solution on initial phasic (open circles) and late tonic (filled circles) component of potassium contractures of 6 portal veins. Original recordings exemplified in the right of the figure. Responses to initial control period set to 100 per cent. Vertical bars in the diagram indicate S.E.

o bout 70 per cent of control occurred in the 7 preparations to which glucose was re-administered. The remaining 2 portal veins were kept in glucose-free medium for a total of 5 h (not shown in the diagram)· by the end of that period their contractile activity had decreased to 32 and 25 per cent of the control.

The effects of glucose-free solution on K -contracture are shown in Fig. 8. In 7 muscles, one contracture was elicited every 30 min over a 2-h period in glucose-free solution. It is seen that the tonic part of the response (filled circles) was severely depressed during the glucose-free period, whereas the phasic part (open circles) was rather unaffected.

Dose-response curves for NA after 2 h in glucose-free solution were obtained in ex periments analogous to those performed in hypoxia (see above). The results are shown in Fig. 9 (6 muscles). As in hypoxia, a pronounced and reversible depression of the entire dose-response curve appeared in the glucose-free solution, with no appreciable shift in ED_{50}.

5. *Biochemical changes caused by hypoxia and by glucose elimination*

Biochemical changes caused by hypoxia (10 mmHg) for 20 min or by glucose-free solution for 2 h, respectively were analyzed. Each determination is based on 10 pooled portal veins (see Methods).

The concentrations of phosphocreatine (PCr), ATP ADP AMP glycogen, glucose-6-phosphate, pyruvate and lactate in the control situation and after 20 min of hypoxia are given in Table I. After hypoxia the PCr and ATP levels were reduced to 35 and 67% of the

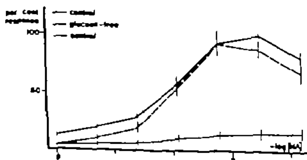


Fig. 9. Cumulative concentration-response curves to noradrenaline for 6 portal veins before (control 1), during, and after (control 2) exposure to glucose-free solution. Results expressed as in Fig. 5.

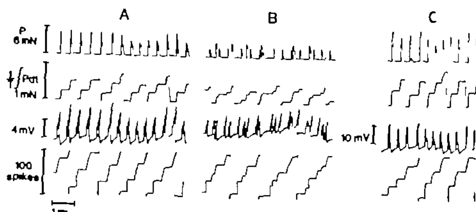


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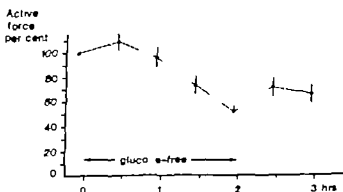


Fig. 7. Change of spontaneous mechanical activity during 2 hrs exposure to glucose-free solution (9 portal veins) and partial recovery after readministration of normal medium (7 preparations). Integrated active force of each muscle in initial control period set to 100 per cent. Vertical bars represent S.E.

-xpts. We have considered two possible explanations for this gradual decrease in spontaneous contractile activity with increasing degrees of hypoxia. The first possibility implies hat each individual cell maintains normal contractile force when its P_{O_2} approaches zero and drops out at the most extreme degrees of hypoxia. The beginning decline of overall contractile force at an oxygen tension of 50 mmHg in the bath would then occur because the cells of the innermost layers of tissue reach a critical oxygen tension due to the O_2 consumption of the outer parts. More and more cells would fail to contract with further decrease in P_{O_2} until finally also the surface layers of the preparation become anoxic at the lowest P_{O_2} levels in the bath. The second alternative implies that spontaneous activity is maintained through some cellular process which itself depends on P_{O_2} in a graded manner at levels 50 mmHg.

Calculations based on values for the rate of O diffusion through vascular tissue (Kirk et al. 1955. Pittman and Duling 1973) and data for O_2 consumption of portal vein (Hellstrand 1977) indicate that the difference in P_{O_2} between the surface and the innermost layer of the preparation, a distance of about 0.06 mm, would be only about 9 mmHg. The first of the above explanations of the oxygen dependence therefore does not seem likely at all. The alternative explanation, considered above, appears to receive some support from the electrophysiological expts. As P_{O_2} was decreased the "pacemaker potential" preceding the bursts became less steep and the number of spikes per burst decreased (Fig. 1). The temporal correlation between electrical and mechanical activity remained, however, suggesting that conduction from cell to cell was still functioning. It appears therefore that graded hypoxia affects impulse generation in the smooth muscle so that the bursts occur at lower frequency and the individual burst encompasses fewer and fewer spikes. Similar observations have recently been reported by Gurevich, Bershtein and Evdokimov (1976). The changes in electrical activity will reduce in a graded way the integrated force developed during spontaneous contractions. Reduced amplitude of spontaneous contractions of dog mesenteric vein in response to anoxia has been reported by Vanhoutte (1976).

Several different mechanisms may be considered as explanations for the mechanical and electrophysiological effects of hypoxia. It is possible of course that decreased ATP availability interferes with ion transport mechanisms essential for normal membrane function including spontaneous impulse generation. Table I shows that both phosphocreatine and ATP decreased in portal vein at a severe degree of hypoxia (10 mmHg). A correlation between PCr and tension at graded levels of hypoxia was demonstrated in rabbit aorta by Namm and Zucker (1973) but they found no decrease in ATP until P_{O_2} was 14 mmHg. Changes in PCr without concomitant shifts in ATP should not be taken directly as an index of altered cellular energy state since the creatinephosphokinase reaction depends on pH and [Mg^{2+}] both of which may change during hypoxia. Our results showing a 33 % reduction of ATP in the portal vein at P_{O_2} 10 mmHg are consistent with those from rabbit aorta (Namm and Zucker 1973) but do not provide information on energy stores at intermediate P_{O_2} levels.

However the effects of hypoxia on electrical and mechanical activity may not be directly related to the decrease in oxidative phosphorylation. It is conceivable, for instance, that the change in membrane activity described above may be caused by decreased intracellular pH secondary to enhanced lactic acid production. The increase in ADP and 5'AMP with the

TABLE I The concentration of phosphocreatine (PCr), ATP ADP AMP glycogen, glucose-6-phosphate pyruvate, lactate and calculated ATP/ADP ratio in the rat portal vein in normoxic conditions and after 20 min of exposure to anoxia The values (mean ± S.E) are given i μmol/g wet c weight Each determination based on 10 pooled portal vein.

	PCr	ATP	ADP	AMP	ATP/ADP	Glycogen (as glucose)	Glucose 6-phosphate	Pyruvate	Lactate
Control n = 4	3 02 ±0.36	2.47 ±0 09	0.612 ±0 046	0.126 ±0.012	4 08 ±0.21	5.07 ±0.23	0 059 ±0 010	0 087 ±0 010	[illegible]
Anoxia n = 4	1.07* ±0 05	1 65 ±0 13	0.901 ±0.042	0.404 ±0.029	1 83 ±0 08	1.83 ±0.37	0.0_3 ±0.003	0 046 ±0 007	[illegible]

p < 0 05 p < 0 01 p < 0 001 based on Student s t test.

respective control values The increases in ADP and AMP did not quite compensate for the decrease in ATP and lowering of the sum of adenonucleotides thus occurred during hypoxia. The levels of alanine, aspartate, glutamate and glutamine did not change (not included in the table) Glycogen, glucose-6-phosphate and pyruvate were all significantly decreased The lactate values varied considerably It is shown in the concomitant paper (Hellstrand 1977) that lactate production in portal vein increases during hypoxia.

In the glucose-free, oxygenated (16%) medium, PCr ATP ADP and AMP remained at control values The levels of glycogen, glucose-6-phosphate, pyruvate and lactate were decreased after 2 hours in glucose-free solution as shown in Table II The amino acids alanine and glutamine were not altered but aspartate and glutamate changed in a reciprocal way

Discussion

Hypoxia

The present expts. have shown that the spontaneous contractile activity of the smooth muscle in rat portal vein is rapidly inhibited in a graded manner when P_{O_2} decreases below 50 mmHg. In this limited range an approximately linear relation between P_{O_2} and myogenic activity was found (Fig. 3) Activity was completely inhibited at the lowest P_{O_2} levels in mos

TABLE II The concentration f glycogen, glucose-6-phosphate, pyruvate, lactate, aspartate, glutamate glutamine and alanine the rat portal vei ncubated in normoglycemic nd glucose-free media for 2 h. The values (means ± S.E.) are expressed i μmol/g wet cell weight. Each determination based on 10 pooled portal veins. Significance levels as Table I.

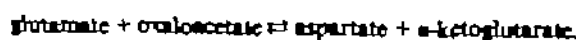
Medium	Glycogen (as glucose)	Glucose-6-phosphat	Pyruvat	Lactat	Aspartat	Glutamate	Glutamine	Alanine
Glucose n = 4	6.56 ±0.48	0.057 ±0 008	0 150 ±0.011	0.60 ±0 06	4 07 ±0 51	7 66 ±0.59	0.43 ±0 2_	0 63 ±0 09
Glucose-free = 4	3 13 ±0.13	0.027 ±0.005	0.065 ±0.015	0.19 ±0 05	7 92 ±_0	5 30* ±0._6	0.91 ±0.38	0 68 ±0 11

the in vitro preparation and that by extrapolation to the dimensions of small precapillary —sels no P_{O_2} effect on contractility was likely. However our results from the portal vein indicate that diffusion limitation is not a probable reason for the oxygen sensitivity of the spontaneous activity in this vascular smooth muscle, which is clearly apparent also at P_{O_2} well above 20 mmHg. It is of interest in this context that cerebral hyperemia due to hypoxia increases markedly below a P_{O_2} of 50 mmHg (Jóhannsson and Siesjö 1974).

Substrate depletion

While the influence of anoxia on the spontaneous activity of the isolated portal vein was rapid and dramatic, removal of the exogenous substrate had rather moderate effects also after prolonged periods (Fig. 6). The sucrose-gap expts. showed mainly a change in the pattern of electrical and mechanical activity indicating desynchronization within the preparation. Burst discharge appeared to originate from more than one "pacemaker" in glucose-free medium and propagation was impaired. Thus, whereas impulse generation seemed to be markedly sensitive to hypoxia, intercellular conduction appeared particularly affected by lack of glucose. This electrophysiological effect of the glucose-free solution may reflect a specific membrane function of glucose or it could be secondary to the changes in cellular metabolism which occur. For instance, it is conceivable that the increased utilization of endogenous amino acids in glucose-free medium could lead to production of substances with special influences on the smooth muscle activity.

Since the muscle can function for several hours in substrate-free medium, although with a graded decline in mechanical activity (Fig. 6), associated with a declining oxygen consumption (Hellstrand 1977), it can probably utilize endogenous substrates for its energy supply. Since there is no lactate production in glucose-free solution (Hellstrand 1977) and since the glycogen stores are not depleted after 2 h in glucose-free medium (Table II), carbohydrate is not a likely energy source in this situation. The possibility of amino acids as endogenous energy supply was considered. Thus there was a decrease of the glutamate and an increase of the aspartate contents. These changes are compatible with a shift in the aspartate-aminotransferase reaction.



glutamate + oxaloacetate ⇄ aspartate + α-ketoglutarate.

Thus, reduction of the pyruvate pool, secondary to the glucose depletion, would induce relative increase in oxaloacetate which causes a shift of the equilibrium to the right. The α-ketoglutarate formed is utilized in the Krebs cycle.

The energetic importance of amino acid catabolism in the portal vein under substrate free conditions cannot be definitely ascertained from the present data. In substrate-depleted rabbit aorta, Coe, Detar and Bohr (1968) were not able to restitute contractile ability by giving glutamate in the bath. There is, however a recent report on metabolization of labelled amino acids in the swine aorta, both in the presence and absence of external glucose (Morrison *et al.* 1976). Bierman *et al.* (1974), in study of cultured rat aortic smooth muscle cells, found a very slight breakdown of the protein moiety of ingested lipoprotein whereas the lipids were utilized. The present results give no indication as to the possible utilization of cellular lipids, for instance from breakdown of membranes, in the portal vein. This potential

575

fall in ATP/ADP ratio (Table I) is consistent with acceleration of glycolysis. Indee fold increase in lactate production was found in hypoxic portal veins (Hellstrand This implies an increased carbohydrate utilization despite the elimination of oxidati sumption. Glycogen present in the portal vein in the control situation would suffice f about 10 min of hypoxia if it were the only carbohydrate source. Several factors rate limiting for the anaerobic energy supply such as rate of transport of glucose i cell limited glycolytic enzyme activity and decreasing intracellular pH It was fo Namm and Zucker (1973) that elevated levels of glucose in the incubation medium in tension development in rabbit aorta under hypoxia. We have not been able to rep these findings in the portal vein by elevation of the glucose concentration to 30 mM however that our control solution had a higher glucose concentration than that Namm and Zucker

Other mechanisms, not directly involved in energy production, may also be consid explanations for the response of the smooth muscle to hypoxia. The inhibition o mediated by specific substances such as prostaglandins and adenosine which may duced in increasing amounts at low P_{O_2}. Cyanide poisoned rabbit aortae, contra noradrenaline, were found by Coburn, Grubb and Aronson (1976) to relax in resp *reduced P_{O_2} indicating that O can exert effects unrelated to its use in mitochondrial* lism.

The present results with K depolarization and noradrenaline (Fig. 4 and 5) show portal vein, although it may have ceased to contract spontaneously can still recruit for active force development at extreme hypoxia if given a sufficient stimulus. A com of Fig. 3 and 4 indicates that the O dependence of the contractile force may ex higher levels of P_{O_2} during a strong excitatory response than during spontaneous Membrane excitation is eliminated by K depolarization and the O dependence un condition is therefore likely to be mediated through some intracellular process, for limitation of the supply of energy to the contractile machinery itself The differenc slope of the regression line for data points below 50 mmHg in Fig. 3 and 4 show greater O sensitivity of the membrane mechanisms discussed above makes O co myogenic vascular activity particularly effective

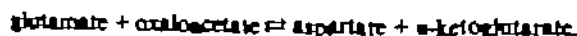
An earlier study by Carrier *et al* (1964) showed that the spontaneous tone of sm teries in vitro is inhibited by low P_{O_2}. Their study and the present one are therefore co with the idea that the important myogenic component of tone in resistance and prec sphincter vessels may be controlled in vivo by variations in the local oxygen tension low range. The problem whether the smooth muscle of these vascular sections exposed to P_{O_2} values of this magnitude during functional hyperemia or autoreg responses cannot, of course be resolved by in vitro experiments. Duling and Berne found that in vivo perivascular P_{O_2} in hamster cheek pouch varied from 35 mmH small arteries to 20 mmHg at the end of terminal arterioles. Little difference was between intra and extraluminal P_{O_2}. *Similar values were reported for the cremaster* from hamster and rat Although in vitro contractile responses of large vessels to adr stimulation shows a graded sensitivity to P_{O_2} in the range < 100 mmHg (Detar an 1968) Pittman and Duling (1973) concluded that this effect was due to diffusion lim

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energy source may however be quite important when the tissue is deprived of exoge substrate supply

NA and K responses of dog mesenteric vein in Vanhoutte's (1976) experiments not significantly reduced after 30 min in glucose-free solution but were markedly depr by the additional influence of anoxia. In the present experiments on the rat portal glucose depletion over 2 had a profound effect on NA and K responses. The fact spontaneous contractile activity was only moderately affected may indicate that endoge substrates can cover the metabolic needs of low level contractile activity reasonably but become quite insufficient when the muscle is challenged by strong excitatory stin The fact that the late component of the potassium responses suffered most seems to f with this line of reasoning

Thanks are due to Mrs Monica Lundahl, Monica Heidenholm and Ina Gunnarsson for able tech assistance. We also want to thank the personnel of Brain Research Laboratory University Hospital L The study was supported by grants from the Swedish Medical Research Council (04X-00028), the Me Faculty University of Lund and AB Hässle, Göteborg.

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as been shown to decline with age in rats (Elsas *et al.* 1971 Fricke and Clark 1973). The esponses of developing diaphragm muscle to denervation may not be comparable to those of adult muscles (Stewart 1968 Asplund 1975).

Methods

rial

Sprague-Dawley rats were used throughout. The animal material comprised 4 age groups, 11 1 and 31-day-old rats (Table I). The two older age groups were given food and water *ad libitum*. The ger animals were nursed by their dams.

ment of animals

animals left thoracotomy was undertaken under ether anaesthesia, and the left phrenic nerve was ed just above the diaphragm. The unoperated right side was left as control, as well as the left side of sham-operated rats. Rats were killed by decapitation 1 to 4 days after the operation. The intact whole ragms were excised as described by Kipnis and Cori (1957). Special care was taken to keep the amount tradiaphragmatic tissue to minimum without damaging the diaphragm muscle fibres. The excised ragms were briefly rinsed with cold Krebs-Ringer bicarbonate buffer (pH 7 4) before incubation. The 31-day-old rats were injected with dactinomycin (0.7 mg/kg b. w.) concomitantly with the ectomy. In these animals the incubations were carried out 2 days after the operation, since the general tion of the dactinomycin-treated rats deteriorated very fast on the third day. Usually the animals 10 to 15 of their body weight.

ation of AIB uptake

intact diaphragm preparations (wet wt about g) were incubated in 2.5 ml of Krebs-Ringer bonate medium (pH 7 4) (Umbreit *et al.* 1972) containing 10 mmol/l glucose and equilibrated with 5 O_2–CO_2 The diaphragms were preincubated for 15 min at 37°C under shaking (110 oscillations/ amplitude 40 mm). After preincubation the diaphragms were transferred to a fresh medium containing additionally 0 5 mmol/l [^{14}C] AIB (50 μCi/l) and incubated for 1 h as above. At the end of incubation the right and left hemidiaphragms were dissected free from surrounding tissue, blotted lightly on r paper, frozen in liquid nitrogen and weighed. Each hemidiaphragm was extracted with 1 ml 5 (/v) containing 5 mmol/l unlabelled AIB for 10 min in boiling water bath. Extracts were centrifuged for n at 730 Samples from the TCA supernatants and incubation media taken before and after incubation were mixed with 10 ml of Instagel scintillation fluid and counted with LKB-Wallac 81000 liquid tillation spectrometer operated with an efficiency of 90 for unquenched standards. Quenching and efficiency of counting were determined by the channel ratio method using an external standard. Each ple was counted long enough to reduce the sample standard error to below 1 .

Table I Effect of left phrenicectomy on the wet weight of the hemidiaphragm 3 days after denervation

of	Number of rats	Body weight[a] g	Right hemidiaphragm[a] (innervated) mg	Left hemidiaphragm[a] (denervated) mg	Difference weights[a]	Statistical analysis[b]
	41	24 9±0 6	28 0±0.7	33 2±0.9	28 4±1 8	P 0 001
	34	50 3 1 0	52.0±1 7	61 6±2.1	18.2±1 4	P 0 001
	36	99 6±1.3	102 9±1.8	128 9±3 0	25.4±1.9	P<0 001
	25	163 5 3 0	156 8±4 0	181 4±5 0	15 8±1 6	P<0 001
	24	172.5 4 5	170 7±3.7	151 9±3.8	13.2±1 5	P 0 001
am rated)						

Mean values with their standard errors
Student' t-test for paired differences.

Acta physiol scand. 1977 100 84–90

From the Department of Biomedical Sciences, University of Tampere, Finland

Denervation Stimulation of α Aminoisobutyric Acid Uptake by the Diaphragm in Developing Rats

By

S C. Mamatas and S S Oja

Received 19 October 1976

Abstract

Mamatas, S C. and S S Oja, *Denervation stimulation of α-aminoisobutyric acid up the diaphragm in developing rats* Acta physiol. scand 1977 *100* 84–90.

Effect of denervation on accumulation of α-aminoisobutyric acid (AIB) by the diaphragm as st 11 1 31 and 51-day-old rats. In both i nervated and denervated diaphragms the accumulation decreased with age. Denervation significantly enhanced accumulation at all ages, the relative in being greater in older animals owing to the lower basal accumulation ratio for AIB. The dene induced stimulatio was partially bolished by dactinomyci administration. The drug obviously the f rmation of new carrier sites at plasma membranes for AIB transport.

K y words. Denervation, diaphragm, development dacti omycin, amino acid transport

Undercutting of one phrenic nerve initially results in a substantial albeit transient in in the wet weight and DNA RNA and protein contents of the denervated part of d phragm (*Sola and Martin 1953 Gutmann et al 1966, Bowman and Martin 1971*). changes are accompanied by an increment in the rates of synthesis of nucleic acids chester and Harris 1968 Zak *et al* 1969) and proteins (Gutmann *et al* 1966, Kimu Kimura 1973 Turner and Manchester 1973 a, Turner and Garlick 1974) The dene diaphragm exhibits acetylcholine supersensitivity (Fambrough 1970) a decreased mem potential (Lüllmann and Pracht 1957) and an enhanced lysosomal activity (Gutmann 1966, Mamatas 1974), *i e* responses typical also for a denervated skeletal muscle. I denervated diaphragm there is an enhancement of intracellular accumulation (Harr Manchester 1966) and an elevation of intracellular levels (Turner and Manchester 1! of a number of amino acids. Similarly denervation effects the uptake of amino aci skeletal muscles (Diehl and Jones 1966, Bombara and Bergamini 1968 Goldberg and (man 1969) The diaphragm thus offers an excellent model for studies on effects of der tion on muscle biochemistry and physiology There have been no attempts to correlat denervation effects on amino acid transport in the diaphragm with the age of experim animals, although the intracellular accumulation of α-aminoisobutyric acid, for inst

...BLE III. Stimulation by denervation of AIB accumulation by hemidiaphragms of 51-day-old rats at varying periods after left phrenectomy. Intact diaphragms ...ere incubated for 1 h in Krebs-Ringer bicarbonate medium (pH 7.4) containing 10 mmol/l glucose under 95 ... 5 ... O_2–CO_2 ...ith 0.5 mmol/l [14C]AIB (50 μCi/l). The accumulation ratio denotes the concentration ratio of AIB obtained between the intracellular water and extracellular medium. Mean values ...ith their standard errors

...ys after ...rectomy)	Accumulation ratio of AIB		Stimulation by denervation	Statistical analysis[b]
	Right hemidiaphragm (innervated)	Left hemidiaphragm (denervated)		
)	0.80 ± 0.07	1.42 ± 0.20	87 ± 15	P 0.005
	0.56 ± 0.04	1.70 ± 0.16	198 ± 14	P < 0.001
	0.63 ± 0.04	2.19 ± 0.25	254 ± 42	P 0.001
	0.68 ± 0.08	1.66 ± 0.13	147 ± 19	P 0.001

...umber of expts. in parentheses
...udent ...-test for paired differences

...ement of the wet weight in the denervated hemidiaphragm exceeds, however, in magnitude ... opposite changes in the relative dry weights, indicating an accumulation of total solids ...ell.

...Denervation significantly enhanced the intracellular penetration of AIB into the dia...ragm in 51-day-old rats (Table III). The effect was already discernible one day after ...ervation and reached its maximum on the third postoperative day. The denervation-...uced enhancement of AIB uptake was therefore systematically studied on the third post...erative day in rats of different ages. In the innervated right hemidiaphragm the accumula...n ratio of AIB was nearly 7-fold greater in 11-day-old rats than in 51-day-old rats (Table ...). Also in the denervated left hemidiaphragm the accumulation of AIB substantially ...creased with the age of the rats. The relativ... magnitude of stimulation by denervation was ...ch more pronounced in older animals, but only owing to the lower basal accumulation ...tio obtained. This denervation-induced enhancement was largely abolished by the injec...n of dactinomycin in the operated rats, whereas the accumulation ratio of AIB in the ...ervated part of the diaphragm was not affected by this drug administration (Fig. 1).

...BLE IV. Stimulation by denervation of AIB accumulation by hemidiaphragms of rats of varying ages 3 days after left phrenectomy. For experimental details see Table III. Mean values ...ith their standard errors.

...e of ...s[a] ...)	Accumulation ratio of AIB Right hemidiaphragm (innervated)	Left hemidiaphragm (denervated)	Stimulation by denervation	Statistical analysis[b]
1 (7)	4.09 ± 0.28	5.80 ± 0.53	41 ± 6	P < 0.001
1 (7)	...36 ... 0.09	4.14 ... 0.23	75 ± 9	P < 0.001
...1 (5)	1.23 ± 0.09	3.31 ± 0.33	152 ± 22	P 0.001
...1 (5)	0.63 ... 0.04	...19 ± 0.25	...54 ± 42	P 0.001

Number of expts. in parentheses.
Student' t-test for paired differences.

TABLE II Dry weights and inulin spaces of hemidiaphragms 3 days after left phrenectomy Intact diaphragms were incubated for 1 h in Krebs Ringer bicarbonate medium containing 10 mm glucose (pH 7 4) under 95 % – 5 % O_2-CO_2 with 0.4 g/l hydroxy [^{14}C] methyl inulin (50 µC for i ulin space estimation For dry weight estimation hemidiaphragms were placed at 12 for 48 h Mean values with thei standard errors.

Age of rats days	Dry weights			Inulin spaces		
	Right hemi diaphragm (innervated) %	Left hemi d aphragm (denervated) %	Statistical significance f dif ferences[b]	Right hemi diaphragm (innervated) %	Left hemi-diaphragm (denervated) %	Statistical significance of dif ferences[b]
11 (7)	1 1±0.3	19 4±0.2	P<0.001	[illegible]8 1±0.4	25.7±0.4	P 0 001
21 (5)	24 6±0.[illegible]	22.[illegible]±0.3	P<0.001	[illegible]0.4±0.3	18.7±0.1	P<0 001
31 (8)	23 6±0.2	1.3±0.2	P<0.001	21.0±0.6	18.4±0.3	P 0 001
51 (6)	[illegible]3.2±0.[illegible]	21.5±0.3	P<0.05	17 1±0.3	17.9±0.5	P 0 1

[a] Number of expts. in parentheses.
[b] Student s t test for paired differences.

Dry eight and Inulin space determination

Excised diaphragm preparations used for dry weight and inul n space determinations ere incubated identically with the preparations used for AIB upt ke measurements only omitting the radioactive AIB. When the i ul n space was determined 0.4 g/l hydroxy[^{14}C]methyl inul n (50 µCi/l) was added to the medium. I ulin preparation (mol. wt. 5 000–5 500) used had been previously purified by gel filtration on Sephadex G-50 as described earlier by Laakso and Oja (1976). For dry weight estimation hemidiaphragms were placed t 120°C u til a constant end weight was obtained (48 h).

Calculation f AIB uptake

Calculations were based on the following assumptions (1) Inul n space represents the extracellular compartment of the diaphragms nd non-i ulin space the intracellular compartment. (2) The difference between the wet and dry weights of the diaphragms after subtraction of the nulin space is equivalent to the intracellular water (3) The concentration of radioactive AIB in the extracellular water of the diaphragm equals the concentration the medium. The tracellular accumulation of AIB was calculated from the total rad oactivity of the slices and from the pecific radioacti ty of AIB n the incubation medium, subtracting the share of inulin space and total solids of the slices. The results are expressed as accumulation ratios of AIB which denote the concentration ratios of AIB between the intracellula nd extracell lar water in the diaphragms. The data were statistically analyzed w th the Student s t test f pa red d fferences

Results

The right-hand part of the diaphragm was in intact rats in all age groups slightly larger than the left hand part when inspected by the naked eye. The wet weights of the hemidiaphragms of 51-day-old sham-operated rats verify this observation (Table I) In contrast, on the 3rd postoperative day the denervated left hemidiaphragm was significantly heavier than the innervated right hemidiaphragm in all age groups A part of this difference may result from accumulation of fluid in tissue since the dry weights of the denervated hemidiaphragms as percentages of the wet weight were invariably less than those of the innervated hemidiaphragms (Table II) Because of the simultaneous reduction in the extracellular (inulin) space on the denervated side the accumulation of water must be predominantly intracellular. The

…BLE III Stimulation by denervation of AIB accumulation by hemidiaphragms of 51-day-old rats 1 … varying periods after left phrenectomy Intact diaphragms were incubated for 1 h in Krebs-Ringer bicarbonate medium (pH 7 4) containing 10 mmol/l glucose under 95 − 5 O_2–CO_2 with 0 5 mmol/l [^{14}C]AIB (50 μCi/l). The accumulation ratio denotes the concentration ratio of AIB obtained between the intracellular water and extracellular medium. Mean values with their standard errors.

…after …ectomy[a]	Accumulation ratio of AIB: Right hemidiaphragm (innervated)	Accumulation ratio of AIB: Left hemidiaphragm (denervated)	Stimulation by denervation	Statistical analysis
[illegible]	0 80 ± 0 07	1 42 ± 0 20	87 ± 15	P 0 005
[illegible]	0.56 ± 0 04	1 70 _ 0.16	198 ± 14	P 0 001
[illegible]	0 63 0 04	2.19 ± 0 25	254 ± 42	P 0 001
[illegible]	0 68 ± 0 08	1 66 ± 0 13	147 ± 19	P < 0 001

…umber of expts in parentheses
…udent t-test for paired differences.

…rement of the wet weight in the denervated hemidiaphragm exceeds, however in magnitude … opposite changes in the relative dry weights, indicating an accumulation of total solids … well.

Denervation significantly enhanced the intracellular penetration of AIB into the dia… phragm in 51-day-old rats (Table III). The effect was already discernible one day after … nervation and reached its maximum on the third postoperative day The denervation-induced enhancement of AIB uptake was therefore systematically studied on the third post… operative day in rats of different ages. I the innervated right hemidiaphragm the accumula… tion ratio of AIB was nearly 7-fold greater in 11-day-old rats than in 51-day-old rats (Table …). Also in the denervated left hemidiaphragm the accumulation of AIB substantially … creased with the age of the rats. The relative magnitude of stimulation by denervation was … uch more pronounced in older animals, but only owing to the lower basal accumulation … tio obtained. This denervation-induced enhancement was largely abolished by the injec… tion of dactinomycin in the operated rats, whereas the accumulation ratio of AIB in the … nervated part of the diaphragm was not affected by this drug administration (Fig. 1).

…ABLE IV Stimulation by denervation of AIB accumulation by hemidiaphragms of rats of varying ages 3 days after left phrenectomy For experimental details see Table III Mean values with their standard errors

Age of rats[a] (…)	Accumulation ratio of AIB: Right hemidiaphragm (innervated)	Accumulation ratio of AIB: Left hemidiaphragm (denervated)	Stimulation by denervation	Statistical analyses[b]
11 (7)	4 09 ± 0.28	5 80 _ 0 53	41 ± 6	P 0 001
11 (7)	2.36 ± 0 09	4 14 ± 0 23	75 ± 9	P 0 001
31 (5)	1.23 0 09	3.31 _ 0.33	152 ± 23	P < 0 001
51 (5)	0 63 _ 0.04	2.19 _ 0.25	254 ± 42	P 0 001

Number of expts in parentheses.
Student' t-test for paired differences.

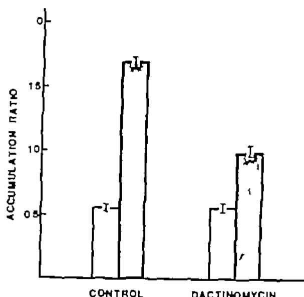


Fig. 1 Effect of dactinomycin on AI[illegible] cumulation by hemidiaphragms of 51-d[illegible] rats days after left phrenectomy The [illegible] (0.7 mg/kg) was injected simultaneously [illegible] the operation. For incubation cond[illegible] see Table III Open columns, right inner[illegible] hemidiaphragms, shadowed columns [illegible] denervated hemidiaphragms. Mean v[illegible] with their standard errors

Discussion

In innervated hemidiaphragms of young rats and in all denervated specimens the accum[illegible] tion ratio of AIB exceeded one, thus revealing the concentrative nature of uptake. Al[illegible] innervated hemidiaphragms of older rats AIB uptake is by nature concentrative, as ca[illegible] demonstrated by using very low concentrations of AIB in the medium or by prolonging [illegible] incubation period (Mamatas and Oja 1976) AIB uptake is evidently a carrier-medi[illegible] process. At physiological pH AIB can be considered a good model substrate for the so-ca[illegible] transport system A specialized for neutral amino acids with a short aliphatic side ch[illegible] (Christensen 1975 Christensen *et al* 1976) The presence and properties of this system h[illegible] also been explored in muscle tissue (Gazzola *et al* 1972, 1973 Franchi-Gazzola *et al* 19[illegible] The present results should therefore be interpreted to show a declining efficiency of th[illegible] system with age and its stimulation by denervation A decrement with age in uptake p[illegible] cesses by tissues appears to be a rather common phenomenon demonstrated for instan[illegible] with glucose and heart muscle (Fricke and Clark 1973) and with amino acids and br[illegible] tissue (Vahvelainen and Oja 1969) At least a part of the enhanced accumulation of am[illegible] acids by foetal and neonatal diaphragm is due to the fast incorporation of free amino ac[illegible] into tissue proteins (Fricke and Clark 1973) On the other hand, the effect of denervati[illegible] on amino acid uptake may be more selective, the system A being particularly affected. F[illegible] example, the uptake of leucine (Buse *et al* 1965) and that of phenylalanine (Harris a[illegible] Manchester 1966) by the diaphragm are not significantly enhanced by denervation wher[illegible] the accumulation of glycine is (Harris and Manchester 1966) Glycine and AIB share t[illegible] transport site A but leucine and phenylalanine are preferably transported by the L syst[illegible] accepting neutral amino acids with a large side chain (Christensen 1975)

There are several ways in which denervation could increase the accumulation of AIB [illegible] the diaphragm, *viz.*, (1) by increasing the affinity of cell membrane carrier sites for AIB, ([illegible] by increasing the total number of carrier sites in the muscle (3) by facilitating the rate [illegible] transfer of AIB across plasma membranes through the pre-[illegible]

ancing the availability of energy for transport if this factor is rate-limiting, or (4) by moshing AIB efflux from the diaphragm. At present we cannot establish whether or not ervation influences the availability of energy or alters AIB efflux from the muscle. Pre-inary kinetic analyses on AIB uptake by the denervated diaphragm suggest that the inity of AIB for carrier sites remains virtually unchanged, but the maximal velocity of nsport increases (Mamatas 1976 a). Dactinomycin, which interferes with the synthesis of v RNA by DNA-dependent RNA-polymerase, partially blocked the denervation-induced ancement. Puromycin and cycloheximide, inhibitors of synthesis of new protein, have a ilar effect (Mamatas 1976 b). It sounds therefore plausible that the total number of rier sites increases during postdenervation hypertrophy of the diaphragm, which signifies her a more dense occupancy of the pre-existing plasma membranes by the newly synthe-ed A transport sites or the formation, through cell hyperplasia or hypertrophy of new sma membranes enriched with those sites.

Dactinomycin, chloramphenicol and cycloheximide ha e been shown to prevent in a nilar manner the development of extrajunctional cholinergic receptors, tetrodotoxin-istant action potentials and the fall in resting membrane potential in denervated skeletal uscle (Grampp *et al.* 1972). A whole number of denervation-induced changes in the muscle re membrane thus pparently depend on the synthesis of new protein. Chronic stimulation denervated muscles with electrical pulses is able to oppose increased uptake of uridine d to inhibit f brillation activity recorded intracellularly (Muchnik and Kotsias 1975). he similarity between the effects of RNA and protein synthesis inhibitors and the effects chronic electrical stimulation in denervated muscles suggests that the normal nerve pulse bombardment exerts regulatory influence on formation of certain membrane omponents through the genome of the muscle cell. The gradual reduction of AIB accumula on with age in developing diaphragm may be similarly related to maturation in the motor ctivity of the phrenic nerves.

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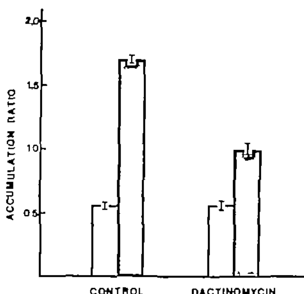


Fig. 1 Effect of dactinomycin on AIB cumulation by hemidiaphragms of 51-da rats 2 days after left phrenectomy The d (0 7 mg/kg) was injected simultaneously the operation. For incubation condi see Table III Open columns, right innerva hemidiaphragms, shadowed columns denervated hemidiaphragms. Mean val with their standard errors.

Discussion

In innervated hemidiaphragms of young rats and in all denervated specimens the accumu tion ratio of AIB exceeded one thus revealing the concentrative nature of uptake. Also innervated hemidiaphragms of older rats AIB uptake is by nature concentrative, as can demonstrated by using very low concentrations of AIB in the medium or by prolonging incubation period (Mamatas and Oja 1976) AIB uptake is evidently a carrier-mediat process. At physiological pH AIB can be considered a good model substrate for the so-cal transport system A specialized for neutral amino acids with a short aliphatic side ch (Christensen 1975 Christensen *et al* 1976) The presence and properties of this system h also been explored in muscle tissue (Gazzola *et al* 1972, 1973 Franchi-Gazzola *et al* 197 The present results should therefore be interpreted to show a declining efficiency of the system with age and its stimulation by denervation. A decrement with age in uptake p cesses by tissues appears to be a rather common phenomenon demonstrated for instan with glucose and heart muscle (Fricke and Clark 1973) and with amino acids and br tissue (Vahvelainen and Oja 1969) At least a part of the enhanced accumulation of ami acids by foetal and neonatal diaphragm is due to the fast incorporation of free amino ac into tissue proteins (Fricke and Clark 1973) On the other hand the effect of denervati on amino acid uptake may be more selective, the system A being particularly affected. F example, the uptake of leucine (Buse *et al* 1965) and that of phenylalanine (Harris a Manchester 1966) by the diaphragm are not significantly enhanced by denervation, where the accumulation of glycine is (Harris and Manchester 1966) Glycine and AIB share t transport site A but leucine and phenylalanine are preferably transported by the L syste accepting neutral amino acids with a large side chain (Christensen 1975)

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Acta physiol. scand. 1977. 100. 91–106

From the Department of Physiology and Biophysics, University of Lund, Sweden

Oxygen Consumption and Lactate Production of the Rat Portal Vein in Relation to its Contractile Activity

By

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Received 21 October 1976

Abstract

Hellstrand, P. *Oxygen consumption and lactate production of the rat portal vein in relation to its contractile activity.* Acta physiol. scand. 1977. *100.* 91–106.

Energy turnover in the isolated rat portal vein was investigated by measurement of oxygen consumption (J_{O_2}) and lactate production (J_{LA}) under simultaneous recording of mechanical activity. In spontaneous activity under aerobic conditions and at optimal muscle length J_{O_2} and J_{LA} were 0.55 and 0.62 μmol/min · g, respectively, corresponding to an ATP-production of 4.3 μmol/min · g. When muscle length was changed, an approximately linear relation was found between energy turnover and mean isometric tension. The tension-independent part of ATP-production was 3.0 μmol/min · g. In Ca^{++}-free solution the metabolic rate was 20% lower still. J_{O_2} was nearly equal in isometric contractions and in afterloaded isotonic contractions from the same initial muscle length. During maximal tonic contracture in K^+-depolarized portal vein J_{O_2} increased to about twice that in spontaneous activity. Changes in contracture force by variations in muscle length or in $[Ca^{++}]_o$ were associated with identical linear relations between J_{O_2} and active tension. This relation was less steep than the corresponding relation for spontaneous activity. The anaerobic lactate production of the portal vein was 2.7 times the aerobic level. The accelerated glycolysis did not compensate for eliminated oxidative metabolism. Under substrate-free aerobic conditions no lactate was produced by the muscle and compared to the control situation J_{O_2} declined more than could be accounted for by reduced mechanical activity alone. The metabolic turnover rate in relation to isometric tension is high in the rat portal vein compared to that of tonic vascular smooth muscle from larger vessels. This correlates with differences in dynamic mechanical properties. At comparable tension levels in the portal vein, the rate of cross-bridge turnover may be higher in spontaneous phasic activity than in sustained contracture.

For the understanding of muscular contraction it is essential to examine how the mechanical performance of the muscle is related to the turnover of metabolic energy. A number of investigations of smooth muscle energetics have been performed on preparations that do not show spontaneous myogenic activity (e.g. Lundholm and Mohme-Lundholm 1965, Peterson and Paul 1974, Stephens and Skoog 1974, Paul and Peterson 1975, Davey, Gibbs and McKirdy 1975, Paul, Glück and Rüegg 1976). Most studies of oxygen consumption in relation to tension of spontaneously active smooth muscles have primarily aimed at revealing mechanisms of pharmacological action (Bulbring 1953, Bulbring and Golenhofen 1967) or

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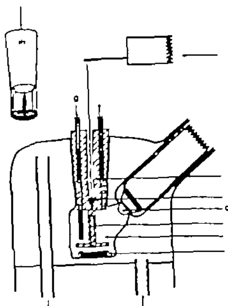


Fig. 1 Apparatus for determination of oxygen consumption of smooth muscle with simultaneous tension recording (inset shows muscle holder a, magnetic stirrer b, measuring chamber, c muscle preparation (hidden), d oxygen electrode, e mercury drop to seal mechanical connection, f perspex muscle holder, inlet tube for perfusion of chamber, h outlet tube, force transducer j, k, tubes for circulating water at 37 C

onds. Force development during the isometric parts of the contractions were recorded by the force transducer which was mounted as afterload stop, and thus supported the load when the muscle was relaxed. Oxygen consumption at the various afterloads was determined over 8–10 min periods.

Measurement of oxygen consumption The apparatus was a modification of the "Combi-Analysator" manufactured by L. Eschweiler & Co, Kiel, Germany. The arrangement is shown in Fig. 1. The muscle (c) was placed in a glass chamber (b) of 1.2 ml volume. An oxygen electrode (d), polarized at 0.6–0.8 V registered the P_{O_2} of the solution in the chamber. A glass-encapsulated magnetic stirrer (a) ensured adequate stirring. The electrode and muscle chamber were contained in a jacket circulated with water at 37 °C from a precision thermostat. The output from the electrode was indicated on a potentiometer recorder (Houston Omniscribe). The muscle was mounted in a perspex holder (f) designed to allow mechanical registration during the experiment. The holder was conically ground to fit into the water jacket as shown in the figure. The lower end of the muscle holder, extending into the chamber, was shaped as a horizontal ring. One end of the muscle was tied to a silver pin fitted in grooves on the lower surface of the ring. The other end was tied to a piece of 6–0 suture silk which passed through an opening in the holder and was connected to the force transducer (). The lowest part of the opening was narrow enough to hold a drop of mercury (e), which served as a seal against leakage of oxygen. It was found that when working at P_{O_2} close to that of air this precaution could generally be omitted, since diffusion of oxygen through the opening was not detectable over the time ranges used for measurements. When both oxygen consumption and lactate production was to be determined a muscle holder was used which contained two additional openings connected to polyethylene tubes (g & h). These were used for perfusion of the chamber with solution as described below. When oxygen consumption alone was to be recorded a muscle holder with one opening for introduction of solutions and drugs was used (not shown). This opening could be sealed by a pin.

The oxygen consumption of the electrode itself was small enough to be undetectable. When the equipment had been turned on for at least 12 h before the experiment drift was negligible, and in no case did it exceed 5 % of the measured oxygen consumption. Since drift, when it occurred, was usually constant over the duration of the experiment it could easily be corrected for. A check for "background" oxygen consumption, was run routinely in each experiment. With fresh solutions containing antibiotics (see above) no background consumption was detected. Before the experiment the oxygen electrode was calibrated with pure N_2 and with the gas mixture to be used. In the aqueous phase the electrode current was about 1 % less than in the gas phase. Oxygen content of the chamber was computed using a value for the solubility coef-

of ionic pump activity (Casteels and Wuytack 1975). A myothermic study of g taenia coli was reported by Mulvany and Woledge (1972)

The rat portal vein differs from many other commonly used smooth muscle pre with myogenic spontaneous activity in that it has a well-defined mechanical resting between the phasic contractions. This makes it easy to identify the active contractile nent of the total tension The force-velocity relation of the portal vein, determi quick release experiments during spontaneous contractions shows a slight curvatu high maximal shortening velocity (Hellstrand and Johansson 1975 cf Murphy 1! cording to the results of Woledge (1968) and of Rüegg (1971) this would indica economy of tension production It was thus considered of interest to examine getic requirements for contraction in this preparation Further portal vein de sustained contracture on depolarization with K^+ high solution and this makes it to compare the energetics of phasic and tonic contraction in the same kind of muscle

The oxygen consumption and lactate production were used as indicators of m rate. The effects of hypoxia or glucose withdrawal on energy turnover in the po were also investigated. The mechanical electrophysiological and biochemical con of these interventions are reported separately (Hellstrand, Johansson and Norberg

A preliminary account of some of the present results has been published (Hellstra

Methods

Sprague-Dawley rats of body weight between 250 and 400 g were used The animals were killed on the neck nd sectio of the portal vein, 6.5–8 mm long, was d ssected o t and freed from f ventitial tissue. The vessels were cut pen longitudinally a d mounted in holder as described b holder was then placed a 50 ml organ bath containing a tris-buffered physiological solution (r as normal tris") t 37°C of the followi g composition in mM N Cl 120, KCl 6 0, $MgCl_2$ 1.2, glucose 11 5 and tris-(hydro ymethyl)aminomethane (Trizma Base, Sigma Chemical C) 35. Th had been t trated with HCl t 37°C t a pH of 7 4 and was bubbled with 100% O_2. The muscle nected to Grass FT-03 force transducer and allowed to equil brate at preload of 5 0 mN f 45 min. Thereafter the gas was changed either to room ai (20 9% O_2) o to 25% O_2–75 N which was used for the nitial co trol period of the e periment A few expts. were performed bonate buffered solution (referred to as Krebs) of the following composition mM NaCl 4 7 $NaHCO_3$ 15.5 KH_2PO_4 1.2, $MgCl_2$ 1.–, $CaCl_2$ 2.5 glucose 11.5 This sol tion was gassed w th co taining 96% O_2–4% CO_2 du g the accommodation period and 80% N_2–16% O_2–4% CO_2 quent periods, respectively givi g pH f 7 3–7 4 at 37°C. K -high tris solution was prepared by 100 mM of NaCl with KCl Nominally Ca^{++}-free tris refers to a solution of the same composit normal solution, except that o $CaCl_2$ had been dded Glucose-free solutions were prepared by glucose a d adding isosmolar amounts of NaCl. All solution were prepared from concentra solutions o the day of the experiment Doubly distilled w ter was used. $CaNa_2$-versenate, 0 026 dded to all solutions t chelate heavy metal ion impurities. To reduce bacterial contamination li G nd streptomycin in concentration of 100 mg/l and 300 mg/l, respectively were added Na salts.

After t least 70 mi t the desired experimental P_{O_2} the muscle was detached from the force tr and transferred on its holder to the measuring chamber (see below) which had previously been f solution. The transducer was agai ttached nd isometric tension, long with ts time i tegral ov periods, recorded on a Grass Polygraph

I one series of expts. the muscle was connected t an isot ic lever (Hell tra d, J hansson ar berg 1972) mounted above the measu g chamber Afterloaded contractions were recorded at

sed. The length was measured with an ocular scale under dissection microscope, whereafter the muscle as cut from its knots, blotted and weighed on Cahn electrobalance. Dry weight as determined after rying in heated evaicator at 200 mmHg and 100°C for 1 h. Dry weight averaged 1 % of wet eight, ith range of 19–23 %. All values for metabolic fluxes are given on he basis of et eight. Cross section as calculated from the wet weight and the length, assuming uniform thickness and density of 1 g cm³

Results

he wall thickness of the rat portal vein is about 0.1 mm and the predominant orientation f the musculature is longitudinal (Johansson *et al.* 1970). The wet weights of the veins used) this study ranged between 1.6 and 3.0 mg. In all expts. reported here, except for those in hich effects of hypoxia were investigated, P_{O_2} was kept above 100 mmHg. At such oxygen ensions the spontaneous activity of the portal vein is essentially independent of P_{O_2} (Hellstrand *et al.* 1977). No difference in either mechanical activity or O uptake was noted in xpts. performed in tris-buffered solutions gassed with 25% O_2–75% N or with air (20.9% O_2), respectively. Therefore these expts. are considered together here.

Effect of length change and of Ca²⁺-free solution

When investigating the contribution of mechanisms responsible for tension development to the total energy turnover of spontaneously active smooth muscle it is important that the pattern of electrical activity does not change appreciably when an altered level of isometric tension is produced. Such a change might cause alteration of activation metabolism. Pharmacological stimulation, e.g. with adrenergic agents, causes a considerable increase in electrical activity of the portal vein (Johansson *et al.* 1967) and thus is not suitable for this particular purpose. Variation of the muscle length appears to be a more satisfactory method. Johansson and Mellander (1975) found that after a 40% elongation of the portal vein from a short initial length the spike frequency was increased by 12 % when the muscle had adjusted to the new length. This static difference in spike frequency is small enough to be negligible in comparison with the large difference in activ isometric tension which is produced by such length change. The middle record of panel A in Fig. 2 shows the active tension developed by the portal vein at a preload of 5.0 mN and a length of 8.7 mm. The top record shows mean tension over 1 min intervals and the bottom record shows, on slower time scale, the oxygen content in the chamber. The chamber was perfused with fresh solution at a constant rate of 4.2 ml/h. It is seen that a constant O content was established within 1 h (see Methods). At the arrow perfusion was stopped and the subsequent linear fall in the oxygen record indicates consumption by the muscle. During the plateau period, before flow was stopped, three successive 1 ml samples were collected for lactate assay. Each sample thus contained lactate produced by the muscle during 12.5 min of activity.

Panel B of Fig. 2 shows the records obtained when the muscle had been shortened by 3.0 mm from the initial length. Preload was now 0.4 mN. The spontaneous activity maintained its basic temporal pattern but mean active tension was considerably lower as compared to the situation show in panel A. At the shorter length less oxygen was consumed per unit time. I panel C the perfusion solution had been changed to nominally Ca -free tris. The muscle now developed no mechanical activity at all. The length of the muscle was the same as in panel B, but oxygen consumption was still lower

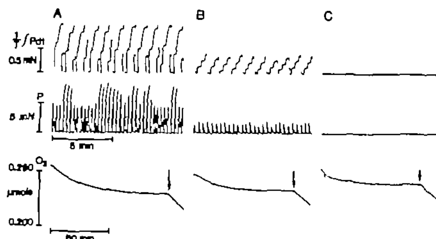


Fig. 2. *Isometric tension and O registration in spontaneous activity of portal vein. Upper record* integrated active tension in 1 min intervals. Middle record active tension. Lower record O_2 content of chamber. Not different time scales. At arrows perfusion was stopped (see text). A Muscle length 17 mm preload 5 0 mN normal tris. B Muscle length 5 7 mm, preload 0.4 mN normal tris C Muscle length 5 7 mm, preload 0 4 mN nominally Ca^{++}-free tris.

ficient at 37°C of 0 0240 ml O_2 (STP)/ml solution (Umbreit *et al* 1957) No measurements of oxygen consumption were made before the muscle had equilibrated in the chamber for at least 20 min. Solution in chamber was changed with 15–30 min intervals. Usually a measuring period of 10 min was adequate determination of the oxygen consumption.

Measurement of lactic acid production. In preliminary experiments it was found that drawing samples from the measuring chamber was not a satisfactory method for determination of lactate production great variability occurred in serial determinations. For this reason a perfusion procedure was adopted means of constant-flow peristaltic pump (Sage 375 A) the chamber was perfused with fresh solution steady rate (in the range 4–6 ml/h). The solution was brought to 37°C before entering the chamber by letting it pass through a coil of tubing submersed in an organ bath filled with bubbled solution and mounted immediately above the inlet to the measuring chamber. Solution left the muscle chamber by means of tube and was collected in 1 ml portions for lactic acid assay. Flow rate was monitored by a drop recorder at the outlet. In some experiments the pump was placed on the outflow side instead, so that solution was drawn directly into the chamber from the bottom of a preaeration reservoir via a short piece of tubing. In this way the inflowing solution could be kept at very low P_{O_2} when desired, since oxygen leakage through the short piece of tubing was insignificant. Temperature changes around the flowing solution would tend to lead to bubble formation. This was avoided by mounting the whole arrangement inside a box kept at 37

The time needed for equilibrium to be established in the chamber can be calculated as follows. Suppose that the muscle produced the metabolite at steady rate x, the volume of the chamber was V and rate of flow of solution through the chamber φ. Let y be the concentration difference between outflow and inflow. Since the contents of the chamber were well stirred and the flow rate was very low it was assumed that the concentration of metabolite in the outflowing solution was the same as that in the chamber. Then $V \cdot dy/dt = x - y \cdot \varphi$. *This represents an exponential approach to the asymptotic* value $y = x/\varphi$ with time constant φ/V. With $V = 1$.. ml and $\varphi = 5$ ml/h the time constant was thus 4.2 h corresponding to a half time of 10 min. Note that the rate for attainment of equilibrium is independent of the actual rate of production of metabolite and that the analysis is valid both for lactate production and for oxygen consumption, in which latter case x takes a negative value. One may therefore presume that the steady-state lactate concentration *in the chamber has been reached when the P_{O_2} registration has stabilized* at a constant value. In the expts. reported here 1 h was allowed for equilibrium, and thereafter 3 successive samples 1 ml each were collected and assayed for lactate in a Zeiss spectrophotometer by the enzymatic method of Hohorst (1970). In principle, from the above calculation, oxygen consumption of the muscle could be obtained from the equilibrium oxygen concentration, but a more accurate determination was obtained by stopping the flow after *collection of the last sample, sealing the inlet and outlet tubes, and recording* the fall of oxygen tension within the chamber over the next 5–10 min, as shown in Fig. 2.

At the end of the experiment the holder was removed from the chamber while keeping the muscle length

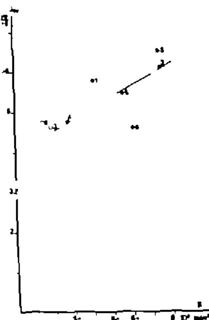


Fig. 4

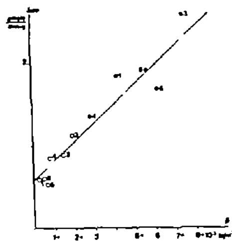


Fig. 5

Fig. 4. Oxygen consumption (J_{O_2}) *vs.* mean active force (P) of portal veins. Filled circles: preload 5.0 mN. Open circles: preload 0.4 mN. Cross: mean ± S.E. of J_{O_2} in Ca^{++}-free solution. Cf. situations A, B and C, in Fig. 3. Number beside each point refers to individual experiment. 6 expts. Broken line fitted by linear regression, r = 0.86. $J_{O_2} = 0.39 + 2.7 \cdot P \cdot 10^{-4}$ in units given on axes.

Fig. 5. ATP-production above that in Ca^{++}-free solution (ΔJ_{ATP}) *vs.* mean spontaneous activity (P). Calculation performed as explained in text. Same experiments as in Fig. 3 and 4. Broken line fitted by linear regression, r = 0.96. $\Delta J_{ATP} = 0.57 + 2.3 \cdot P \cdot 10^{-4}$ in units given on axes.

long and short length, respectively. The resulting quantity, termed ΔJ_{ATP}, is plotted against active tension. This procedure, to some extent, reduces the variation between expts. The intercept of the regression line on the ordinate (0.57 µmol ATP/min · g) might be interpreted as the metabolic cost of Ca^{++}-dependent activation processes.

The calculated total ATP-productions corresponding to the values given in Fig. 3 are: at long length (A) 4.3 ± 0.[illegible] µmol/min · g, at short length (B) 3.2 ± 0.2 µmol/min · g and in Ca^{++}-free solution (C) 2.4 ± 0.1 µmol/min · g.

If length change per se has an effect on metabolic flux regardless of altered tension levels the above analysis would be invalidated. However, in control experiments oxygen consumption was determined at different muscle lengths in Ca^{++}-free solution and was found to be uninfluenced by the length changes.

Oxygen consumption during tonic activity

In 5 expts. oxygen consumption of the portal vein was determined during contractures of 10 min duration obtained by the addition of Ca to the muscle while it was depolarized by K-high Ca^{++}-free solution, as described by Hellstrand, Johansson and Ringberg (1972).

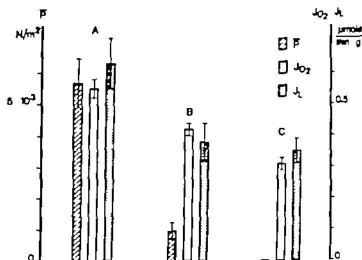


Fig. 3. Mean active tension ($\bar{P}$), oxygen consumption (J_{O_2}) and lactate production (J_{LA}) of portal vein. Explanation of symbols shown in inset. Means ± S.E. of 6 expts. A. Spontaneous activity at near optimal length for active force development (preload 5.0 mN). B. Spontaneous activity of shortened muscle. Preload 0.4 mN. Muscles shortened 36 ± 3 % (S.E.) as compared to length in A. C. Muscles in Ca^{2+}-free solution. Same length as in B.

Fig. 3 shows collected results from experiments of this kind performed on six muscles. Panels A, B and C correspond to the situations shown in Fig. 2. The length changes were on the average 36 ± 3 % (S.E.) of the initial muscle lengths. Mean active tension developed by the muscles is expressed as tension per cross-sectional area. It is seen that both oxygen consumption (J_{O_2}) and lactate production (J_{LA}) decrease as active tension decreases, and that, furthermore, in all three situations J_{O_2} and J_{LA} are of comparable magnitude in molar quantities. The correspondence between tension development and energy turnover is further demonstrated in Fig. 4, which shows oxygen consumption of the individual muscles plotted against mean tension. The points obtained at long muscle lengths are shown as filled circles, those at short lengths as open circles. The numbers beside the points correspond to the individual experiments. The mean and S.E. of oxygen consumption in Ca^{2+}-free solution is shown by the x symbol. Considering that the diagram compiles data from six individual experiments the rather wide scatter may be expected. Nevertheless a clear correlation is demonstrated. The broken line is fitted by linear regression. Note that lines combining the two points in each experiment mostly have slopes rather close to that of the regression line, although the absolute positions in the vertical direction vary. The intercept of the regression line on the vertical axis is clearly different from the J_{O_2}'s obtained in Ca^{2+}-free solution (see also Table I). This difference may represent oxygen consumption needed to maintain electrical activity and excitation-contraction coupling in the cells in normal ionic environment, since the portal vein is electrically quiescent in Ca^{2+}-free solution (Axelsson *et al.* 1967).

A calculation of the total metabolic cost of tension development in terms of creation of high-energy phosphate bonds has been attempted in Fig. 5, where the conventional stoichiometric coefficients of 6.42 mol ATP/mol O and 1.25 mol ATP/mol LA were used (*cf.* Peterson and Paul 1974 and see Discussion). For each preparation calculated ATP production in Ca^{2+}-free solution was subtracted from ATP production during spontaneous activity at

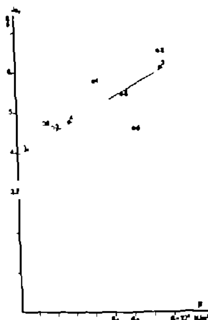


Fig. 4

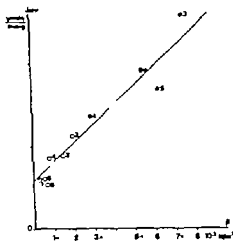


Fig. 5

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long and short length, respectively. The resulting quantity, termed ΔJ_{ATP}, is plotted against active tension. This procedure, to some extent, reduces the variation between expts. The intercept of the regression line on the ordinate (0.57 μmol ATP/min · g) might be interpreted as the metabolic cost of Ca^{++}-dependent activation processes.

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TABLE I *Oxygen consumption of the rat portal vein in spontaneous activity and in K -contractures.* the linear regression of J_{O_2} on $\bar{P}$ was calculated the regression coefficient, $\Delta J_{O_2}/\Delta\bar{P}$ and the ten independent portion of the oxygen consumption, obtained as extrapolated J_{O_2} for P 0 L normal" and K high Ca^{++}-free solution are also shown p-values based on Student's t for unpaired data.

Experimental conditions	$\Delta J_{O_2}/\bar{P}\Delta$ $\frac{\mu mol}{min \times g}\left(\frac{N}{m}\right)^{-1}$	J_{O_2} at P = 0 μmol/ min × g	p	J_{O_2} at $[Ca^{++}]_o$ = 0, μmol/min g		
				normal" tris	p	K -high tris
Spontaneous activity Altered length	(-9 ± 0.4) 10^{-4}	0.39 ± 0.01	< 0.0025	0.31 ± 0.02		
p	< 0.025	< 0.025		> 0.20		
K^+-contracture, Altered length	(1.8 ± 0.3) × 10^{-4}	0.51 ± 0.05	< 0.0025	0.34 ± 0.01	> 0.98	0.34 ± 0.01
p	> 0.80	> 0.40		> 0.60		0.60
K -contracture, Altered $[Ca^{++}]_o$	(1.8 ± 0.1) × 10^{-4}	0.47 ± 0.02	< 0.0005	0.33 ± 0.01	> 0.80	0.33 ± 0.01

The Ca^{+} -ions were added in 0.12 ml tris solution to give final concentrations in the cham of 0.05 0.2, 0.4 0.8 2.0 and 11.0 mM respectively without alteration of tonicity Th concentrations of Ca produce *sustained contractures of graded strength (cf dose-respo* curves in Hellstrand *et al* 1972) In Fig. 6 is shown a linear regression of the oxygen c sumption against active tension for each individual contracture. Note that the scale on *horizontal axis is different from that in Fig. 4 The responses obtained with the lo* Ca -concentrations (0.05 mM) are shown as open circles (4 expts.). These points are included in the regression Oxygen consumption in Ca^{++} free K high solution is indic on the ordinate. *In 6 further expts. graded* K -contractures were *obtained instead* standard [Ca] of 2.5 mM but with the length of the muscle varied to produce diffe degrees of active tension Table I compares the regression lines for J_{O_2} vs. mean ten obtained in the expts on spontaneous activity and on K -contractures *with variatio* length and of [Ca^{+}], respectively For each group of expts. the means ±S.E. of the reg sion coefficients and of the extrapolated J_{O_2} at P = 0 is tabulated For the case of the sp taneous activity the regression *line* for an *individual* muscle had to be *constructed fr* two points only (at long and at short length respectively), whereas for the contractures points were included in each regression

The unit for the regression *coefficient* used in the table and *in the figure caption* (μmol/min g) (N/m^2) Notice, however that in the present work the cross-sectional area computed as weight/length of the muscle, assuming a density of 1 g/cm Thus the calcula slope is independent *of the actual weight of* the muscle and the unit above *may equivalen* be written as mmol/N m min The results in Table I show that the slope of the regress line for spontaneous activity is significantly greater than the slopes obtained for K -co tractures, either with altered *length or altered [Ca] The extrapolated* J_{O_2} *at P = 0* lower in the spontaneously active muscles However in Ca -free solution all 3 grou

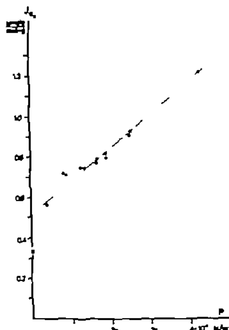


Fig. 6. Oxygen consumption (J_{O_2}) in K -contractures plotted against contracture tension (P). Data from 6 expts. Note change of scale on force axis as compared to Fig. 4. Graded contracture force obtained by varying $[Ca^{++}]_o$ as explained in text. Points obtained with lowest Ca^{++}-concentration (0.05 mM) shown as open circles. Cross symbol shows J_{O_2} in Ca^{++}-free solution (±S.E. within size of symbol). Dotted regression line based on points with $[Ca^{++}]$ > 2 mM (filled circles), 0.95 J_{O_2} = 0.51 + 1.6 · P · 10^{-4} in units given on axes.

showed similar J_{O_2}. There was likewise no difference between J_{O_2}s measured in "normal" and K -high Ca -free solution.

Oxygen consumption during performance of external work

Afterloaded contractions were recorded during spontaneous activity at preloads ranging between 2.0 and 5.0 mN (8 expts.). It was found that at a given initial length the rate of oxygen consumption was very nearly the same whether or not the muscle was allowed to perform external work. J_{O_2} in isotonic contraction was not consistently greater or smaller than in isometric contraction at the same initial length. Since the effects of shortening on J_{O_2} were very small no attempt was made to rigorously correlate external work performance with total enthalpy change. Such an analysis would require more regular activity than that of the spontaneous contractions.

Effects of hypoxia on metabolic flux

As shown in the concomitant paper (Hellstrand, Johansson and Norberg 1977), hypoxia causes a reduction both of electrical and mechanical activity in the portal vein. A partial depletion of the stores of high energy phosphate compounds occurs after anaerobic incubation for 20 min. In the present work oxygen consumption and lactate production in extreme hypoxia were determined in expts. analogous to those described above. Fig. 7 summarizes the results of 6 expts. A severe reduction of mechanical activity occurred in hypoxia. In no expt. was any oxygen consumption measured in hypoxia (mean P_{O_2} 3.5 mmHg). It is therefore probably accurate to refer to this situation as *anoxia*. On the other

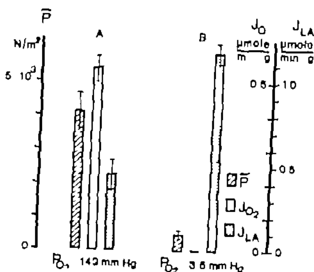


Fig. 7 Mean active tension (P), ox[ygen con]sumption (J_{O_2}) and lactate produc[tion] of portal veins in control medium ( ) [and] anoxia (B). Explanation of symbols [in] inset. Means ± S.E. of 6 expts.

hand lactate production was increased to about 2.7 times the control value On r[eturn to] aerobic conditions, after more than 2 h anoxia, all muscles recovered spontaneous[ly] which transiently had an intensity above the control level.

Oxygen consumption in glucose free solution

The oxygen consumption was followed during a 2 h period of glucose-free incubatio[n] tion was shifted every 30 min The result is shown in Fig. 8 Over the period, a decline in both mean contractile force (P̄) and J_{O_2} occurred Readmission of glucose an incomplete recovery

In 4 expts. the perfusion procedure was used and samples analyzed for lactate a[fter] glucose-free incubation However in none of the samples could a lactate content in of the blank value be found.

J_{O_2} was compared with P̄ in the control situation and after 2 h glucose-free incu[bation] respectively The results are shown in Table II When the reduction in mechanical a

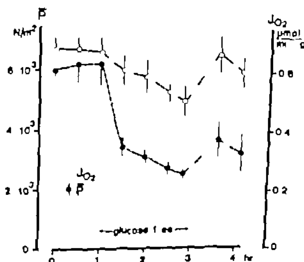


Fig. 8 Spontaneous mecha[n]ical a[ctivity] (filled circles) [an]d oxygen consumption [(open] circles) of portal vei[n]s in control medium d[uri]ng 2 h of exposure t[o] glucose-free [solu]tion. Symbols show means ± S.E. of 4 e[xpts.]

TABLE II. Oxygen consumption in control medium and in glucose-free solution.

	$\bar{P}$ N/m²	J_{O_2} μmol/min g	$\Delta J_{O_2}/\Delta \bar{P}$ $\frac{\mu mol}{min\ g} \left(\frac{N}{m^2}\right)^{-1}$
Control	(3.91 ± 0.43) 10⁴	0.604 ± 0.031	(5.05 ± 0.60) 10⁻⁶ g
Glucose-free 2 hrs	(1.99 ± 0.31) 10⁴	0.419 ± 0.038	

…as caused by glucose removal $\Delta J_{O_2}/\Delta \bar{P}$ was found to be considerably greater ($P < 0.01$) than in the case of spontaneous activity with glucose but at different muscle lengths (*cf* Table I). The energetic difference between these two situations is even more accentuated when the absence of lactate production in glucose-free solution is taken into consideration.

Effect of buffer medium

Tris-buffered solutions bubbled with O_2–N_2 mixtures, or air were used in the above series of expts. It was considered advantageous to have gases free of CO_2 since the CO_2-tension in the measuring chamber could not be recorded. However 4 expts. were performed in Krebs solution bubbled with 80% N_2 16% O_2–4% CO_2. Qualitatively the muscles behaved like those in tris medium. Mean active tension (spontaneous activity) was (4.7 ± 0.5) 10⁴ N/m² and J_{O_2} was 0.48 ± 0.02 μmol/min g. These values are well within the range of those found in tris medium. One consistent observation was that in anoxia the muscles in tris medium maintained some spontaneous activity in the measuring chamber (*cf* Fig. 7), whereas in expts. with Krebs solution, which were performed in organ baths with continuous bubbling, spontaneous activity usually disappeared at P_{O_2} below 5–7 mmHg (Hellstrand *et al.* 1977). The reasons for this difference were not systematically investigated.

Discussion

The rate of metabolism of the rat portal vein was seen to vary with the level of isometric tension (Fig. 2–6). In spontaneous activity at optimal muscle length about 30% of the energy turnover could be ascribed to the contractile activity as such (Fig. 3 and 5). In maximal contractures elicited by stimulation with K⁺-high solution the rates of oxygen consumption were about twice the rates measured during spontaneous activity. Thus the range of variation of metabolic rate between rest and activity is quite narrow in the portal vein when compared to the adaptable energy turnover of skeletal muscle. This is consistent with findings in other smooth muscles (Kosan and Burton 1966, Bülbring and Golenhofen 1967, Stephens and Skoog 1974, Paul and Peterson 1975).

The rate of oxidative metabolism may be expected to reflect the energy utilization by the tissue. It has been noted, however, that vascular smooth muscle often shows a net production of lactic acid even under aerobic conditions (Lehninger 1959, Peterson and Paul 1974). Thus an energetic analysis will have to take account also of the contribution from glycolysis to the total energy turnover. In the present study it was found that at a P_{O_2} corresponding

to alveolar air the portal vein produced lactic acid at a rate which in molar quantities was about equal to the oxygen consumption The energetic importance of the lactate production is dependent on the source of the glucose molecules entering the glycolytic chain. The results presented in the concomitant paper (Hellstrand *et al* 1977) indicate that the glucose utilized by the portal vein is largely derived from extracellular sources. Under this assumption 1 high-energy phosphate bond is created for each lactate molecule formed. Thus approximately 14% of the energy production is derived from glycolysis under aerobic conditions If instead all glucose is assumed to come from endogenous glycogen 20% of the energy production will be derived from glycolysis. Note that in constructing the diagram Fig. 5 the assumption was made that half of the glucose was derived from glycogen. This was done chiefly to enable comparison with other recent studies on smooth muscle (Peterson and Paul 1974 Kroeger 1976 Paul Glück and Rüegg 1976) As in these other studies P/O-ratio of 3 0 has been assumed. In the present work lactate production could be determined only over relatively long periods of steady state activity This precluded determination of glycolytic rate during K -contractures. Therefore, the energetic comparison of phasic and tonic contraction in the portal vein has to be performed here on the basis of oxygen consumption alone leaving open the possibility that a substantial increase in lactate production may have occurred in the contractures. It is not likely however that glycolysis in the contractures would proceed faster than the anaerobic rate (Fig. 7). Not even at this rate would ATP production from glycolysis be large enough to eliminate the energetic differences between phasic and tonic contraction which are illustrated in Table I

Qualitatively the demonstration of a linear J_{ATP}–P relationship in the portal vein is in line with other observations on both smooth and striated muscle (*e.g.* Hill 1958 Dawson Gibbs and McKirdy 1975 Paul and Peterson 1975) The slope of the metabolism vs tension relation in portal vein does however show interesting quantitative differences in comparison with other muscles. Some published results for J_{O_2} in relation to isometric tension in smooth muscle are summarized in Table III Included in the table are also values for "basal" J_{O_2} unloaded shortening velocity (V_{max}) and the constant a/P of Hill's (1938) equation available. All values apply to a temperature of 36–37°C The comparison should be regarded cautiously since the different investigations vary in definition of "basal" conditions and technique for mechanical expts Among the vascular smooth muscles the rat portal vein differs from most larger vessels in having a rather high resting J_{O_2} In addition, the $\Delta J_{O_2}/\Delta P$ relation is markedly steeper in this preparation The higher metabolic rate is accompanied by a considerably higher shortening velocity It is especially interesting that the phasic spontaneous activity of the portal vein seems to be characterized by a higher $\Delta J_{O_2}/\Delta P$ value than the tonic K -contracture. This correlates with findings of a less curved force-velocity relation and a higher V_{max} in phasic contractions than in contractures (Hellstrand and Johansson 1975 and unpublished observations) The possibility arises that maintained tonic contraction somehow results in a diminished rate of ATP-utilization without reduction of the isometric tension. Diminished shortening velocity with prolonged K -contractures has been demonstrated in amphibian slow striated muscle fibres (Lännergren 1976) Whether these effects are specific for K -depolarized muscle or a general phenomenon in prolonged contraction appears not to be known at present. Sustained activity will be expected to

…BLE III. Comparison of oxygen consumption and mechanical parameters at 37°C in some smooth muscles

	Basal J_{O_2} $\frac{\mu mol}{min \cdot g\ (wet\ wt)}$	$\Delta J_{O_2}/\Delta F$ $\frac{\mu mol}{min \cdot g}\left(\frac{N}{m_2}\right)^{-1}$	V_{max} muscle length/s	/P	Ref.
…rotid artery, hog	0.08	0.09 10^{-4}	0.12	0.18	Paul, Glück and Rüegg 1976; Herlihy and Murphy 1973
…moral artery, dog	0.45				Kosan and Burton 1966
…esenteric vein, calf	0.10	0.14 10^{-4}	0.02	0.15	Paul and Peterson 1975; Peterson 1974
…rtal vein, rat					
Spontaneous activity	0.39	2.9 10^{-4}	0.34	0.73	This study; Hellstrand and Johansson 1975
K-contracture	0.49	1.8 10^{-4}	0.4	0.6	This study; Hellstrand, unpublished results
…enia coli, guinea pig	0.45–0.90		0.3	0.17	Bülbring and Golenhofen 1967; Mashima and Handa 1969
…rachealis, dog	0.75		0.17	0.21	Stephens and Skoog 1974; Stephens, Kroeger and Mehta 1969

…iminish the available amounts of high-energy phosphate compounds. As a result, an increased formation of rigor complexes (Weber and Murray 1973) may appear resulting in reduced shortening velocity and more economic tension maintenance.

It may be questioned whether increments in mean spontaneous activity and in contracture tension are quantities that can be meaningfully compared. If active tension is generated by cross-bridges, however as seems to be the case also in smooth muscle (Lowy *et al* 1973 Somlyo *et al* 1973) it follows that tension in the whole muscle is basically an additive quantity determined at each instant of time by the number of attached cross-bridges and by the tension over each individual bridge. Since the rate of bridge cycling is related to the ATP splitting, and thus to the metabolic cost of contraction, the present results indicate that the mean tension per attached cross-bridge should be lower in phasic contraction. This conclusion seems reasonable also on the basis of the shape of the force–velocity relation.

In phasic activity the contractile component of the muscle continually stretches and relaxes the series elasticity. This represents internal work which might require additional turnover of chemical energy. Whether work performed by muscle adds to the total metabolism has been the subject of much debate ever since the original observations on heat production during isotonic contraction in frog sartorius muscle (Fenn 1923). Recently it has been shown that number of factors, such as stimulus conditions, temperature and initial length influence the relation between isometric and isotonic heat production in skeletal muscle (Gibbs and Chapman 1974). In a number of studies on striated muscle, oxygen consumption during isotonic twitch or short tetanic contractions has been found not to exceed that during isometric contraction, except possibly at high loads (Fischer 1931 Whalen 196., McCarter and Ramsey 1968). In the present study it was found that for given initial length the

oxygen consumption of the portal vein was very nearly the same whether the muscle wa isometric or was allowed to shorten against graded afterloads. The reasons for this beha of the spontaneously active muscle are probably quite complex, and the important poi the present purposes is the demonstration that the performance of a certain amou external work does not substantially influence oxygen consumption. Therefore it doe appear likely that internal work under phasic activity contributes so much to the e turnover as to seriously affect the main conclusion, *i.e.* that tension development in p activity is associated with a higher rate of ATP turnover than a comparable active te level in a tonic contracture. An internal compliance will however somewhat reduce th metric tension measured in a phasic contraction. The magnitude of this effect cann accurately determined in the irregular contractions but it is likely that it will be to extent opposed by the prolongation of contraction time that appears in isotonic cond (Johansson and Hellstrand 1975). One other possible source of error in this analysis is activation metabolism may vary for instance with muscle length. This cannot be defi excluded on the basis of the present data but it is notable that the regression lines of J contracture tension were not dependent on whether the alterations in active tension produced by changing muscle length or extracellular Ca -concentration (see Table I). T two procedures would be expected to have quite different effects on excitation-contra mechanisms.

When extrapolation is made to the point where no isometric tension was exerted b spontaneously active portal vein the calculated ATP production was about 3.0 μmol/m (*cf.* Table I). In Ca -free solution ATP production was about 70 % lower. Vascular sm muscle probably possesses an energy-dependent mechanism to keep the intracellular level low in the absence of stimulation (van Breemen 1976). It is interesting that in contractures the J_{O_2} *vs.* P relationship showed a significantly increased intercept on th axis as compared to spontaneous contractions. The continuous depolarization evid causes a massive inflow of Ca -ions which has to be counteracted by the active extr mechanism. In Ca -free solution no difference in oxygen consumption was noted bet "normal" and K -high solution. This lack of a metabolic effect does not necessarily that the activity of the Na -pump is uninfluenced by K -high incubation. It shou noted that the Ca -free state itself may cause depolarization of the cell membrane (A son *et al.* 1967).

At a P_{O_2} of 3.5 mmHg the lactate production of the portal vein was increased to a 2.7 times the control level. At this severe degree of hypoxia no oxygen consumption appe so presumably glycolysis provided all energy for the cell. Depending on the assumptio to the origin of the glucose molecules the ATP production can then be estimated at 1.8 μmol/min g, which is considerably below the value of 3.0 μmol/min g for the ten independent metabolism under aerobic conditions. Evidently other energy depen cellular functions than just the contractile machinery are compromised in hypoxia. instance, the electrical membrane activity is highly sensitive to P_{O_2} (Hellstrand *et al.* 1 On readmission of oxygen after a period of anaerobic incubation the spontaneous con tions usually showed a temporarily increased intensity compared to the control level be the anaerobic period. The cause of this effect might be a redistribution of electric ch

surround the cell membrane as a result of depressed ionic pump activity or increased production of lactic acid during the hypoxic period.

In glucose-free solution the spontaneous activity gradually declined to about 40% of control after 2 h. Concomitantly oxygen consumption was reduced. No lactate production was apparent under glucose-free incubation. Since the glycogen stores of the portal vein are small and, moreover since the glycogen is not depleted under the actual conditions (Hellstrand *et al.* 1977), it must be concluded that substrates other than carbohydrate have been utilized. It is notable that the oxygen consumption declined more than would be expected on the basis of the reduced mechanical activity (Table II). In addition to the fact that there was no glycolysis this indicated that the metabolic activity was considerably depressed in substrate-free conditions although the effect on spontaneous mechanical activity was moderate.

Prof. Börje Johansson has provided helpful encouragement and criticism. Mss Monica Lundahl, Monica Lindenholm and Ise Gunnarsson have assisted ably in the experimental and secretarial work. The study was supported by grants from the Swedish Medical Research Council (04X-00028), the Medical Faculty University of Lund and AB Hässle, Göteborg.

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Acta physiol. scand. 1977. 100. 107–114

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Anaerobic Performance Capacity in Athletes

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Received 19 October 1976

Abstract

KOMI, P. V., H. RUSKO, J. VOS and V. VIHKO. *Anaerobic performance capacity in athletes.* Acta physiol. scand. 1977. *100*. 107–114.

Anaerobic performance characteristics of the whole body and at muscle tissue level were studied in 89 athletes and 31 reference subjects. The main parameters were vertical velocity during running up the stairs, maximal isometric force of leg extensor muscles, blood lactate concentration after maximal treadmill running test, percentage of fast twitch muscle fibers (% FT fibers), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activity in vastus lateralis muscle. These parameters tended to divide the athletes and their sport events into neuromuscular, anaerobic and aerobic types. The specific needs of the different sport events might have modified the expected characteristics of energy and power utilization. However, a high percentage of FT fibers might be a prerequisite for a successful athlete in certain neuromuscular anaerobic type events ("power events"). The main parameters describing the anaerobic performance capacity of the whole body (vertical velocity, leg force, blood lactate) were found to be related to muscle fiber composition (% FT fibers). The running velocity rather than muscle strength seemed to be more influenced by activity of enzymes LDH and CPK.

The energy production for metabolic activities can in general terms be divided into aerobic and anaerobic components. In man maximal aerobic power has been used as a criterion to study the efficiency and performance capacity of the aerobic energy component (e.g. Hollmann et al. 1964, Saltin and Åstrand 1967, Gollnick et al. 1972, Bergh 1974). Despite the fact that the role of the anaerobic metabolism at work has been extensively studied in normal man (Karlsson 1971) much less attention has been given to the investigation of its capacity and power in skeletal muscles of different athlete populations. Therefore the purpose of this study was to investigate these aspects on athletes representing sport events of differing need for muscular force, speed and endurance.

Material and methods

Altogether 89 athletes of international or national level and 31 reference persons were selected as subjects. The athlete group represented various sport events as follows: cross-country skiing (17 men and 5 women), nordic combination (5), skijumping (9), alpine skiing (6), speed skating (6), ice hockey (13), canoeing (8), long distance running (8), 800 m running (4) and power events (100–400 m running 3, throwing 1, jumping 1

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yed as muscle biopsies taken according to Bergström (1962) in most cases from m. vastus lateralis) and in some cases also from m. gastrocnemius c.l. (GL) and m. deltoideus (D). For classification of uscle fibers as fast twitch (FT) or slow twitch (ST) types (Gollnick *et al.* 1972) myosin ATPase staining sed according to Padykula and Herman (1955) LDH and CPK activities were assayed in case of men women cross-country skiers, nordic combination skiers, alpine skiers and skijumpers. A small piece 10 mg) by inspection free from blood and connective tissue was rapidly weighed and 2 ,. homogenate 1 M Tris-HCl buffer (pH 7.5) was prepared in glass homogenizator at 0°C. The homogenate was cenuged for 10 min at 930 g and aliquots for protein determination (Lowry 1951), and LDH and CPK ays were separated. LDH determination was performed according to Kornberg (1955). CPK activity s assayed th Biochemica Boehringer test combination. Enzyme activities were expressed as µmol s^{-1} mg^{-1} supernatant protein 22°C. The determinations were performed during the same day when muscle sample was taken.

Results

e means and standard deviations for different variables are shown in Table II. The mean rtical velocity varied from 1 17 m/s for women cross-country skiers to 1.57 m/s for power

BLE II. Means and standard deviations of the different variables studied.

roups	Number of subjects	Vertical velocity m s	Muscular power kgm s^{-1}	Total leg force kg	Relative horiz. force kg kg^{-1} B.W	Blood lactate mM	FT-fib. in m. VL	Enzyme-activities in m. VL (µmol mg^{-1} min^{-1}) LDH	CPK
ower events	6	1.57 0 13	119 8 17 1	365 70	4.78 0 80	13.2 2.8	63 19	—	—
00 m running	6	1 40 0.20	101 8 19 3	294 84	4 06 1 06	13.9 2 1	35 16	—	—
ki-jumping	9	1 43 0.10	99 7 12.4	314 59	4.49 0 59	10 7 1 7	45 8	1.54 0.34	7.96 .43
anoeing	8	1.40 0 05	111 9 9 6	377 26	4 77 0.31	13.3 1	42 9	— —	— —
ce hockey	13	1 53 0 09	117 7 9 6	353 71	4.56 0 86	11.0 1 7	39 12	—	—
Alpine skiing	6	1 43 0 10	100.5 15 0	324 34	4 60 0 32	13 6 1 9	37 8	1 75 0 51	10 02 0 51
Nordic combination	5	1 31 0 09	92.5 [illegible]	294 54	4.17 0 59	11 8 2.1	37 5	1.30 0.30	6 97 1.67
Cross-country skiing, men	17	1 28 0.11	88.6 11.3	293 43	4 24 0 57	11 4 1.5	37 9	1.10 0 36	7.96 1 08
Speed skating	6	1 53 0 14	116 8 12.5	307 48	4.12 0.57	9.0 1 1	31 13	—	—
Long-distance running	8	1.31 0 07	87.1 7.0	250 42	3.77 0.59	11.8 1.8	22 6	—	—
Physical education students	8	1.51 0 08	106 9 15.1	296 57	4 12 0 64	9 7 1.1	49 13	—	—
Control persons	23	1.32 0.13	98.9 16.7	305 71	4 08 0 65	10.8 2.2	54 14	—	—
Cross-country skiing, women	5	1 17 0 07	89 3 7 0	227 63	3.90 1.28	11 0 2.6	40 13	1.07 0 26	7.38 1.54

TABLE I Physical characteristics of the groups studies.

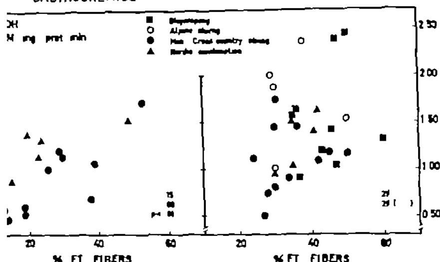
Group	Age yrs	Height cm	Weight kg	FFW[a] kg	Fat [b]
Power events	23 4	176	76.3	63.1	13.0
(n=6)	4 1	5	6.4	5.3	3.4
800 m running	24 6	179	72.3	62.9	12.4
(n=6)	2.2	4	4.6	4.5	1 4
Skijumpi g	22.2	174	69 9	59.7	14.3
(=9)	2.4	5	8.5	5.2	3.7
Canoeing	23 7	182	79 6	68.9	12.4
(n=8)	4.2	5	6.5	4.4	2.8
Ice hockey	22.5	171	77.3	65.3	13 0
(n=13)	3.5	3	5 7	4 7	2.7
Alpine skiing	21.2	176	70.1	60 8	14 1
(n=6)	2.4	6	8 0	5.9	3.0
Nordic combination	22.9	176	70.4	62.0	11.2
(n=5)	2.1	5	5 7	5 0	1 4
Cross-country skiing	25 6	174	69.3	60.3	10..
men (n=17)	3.2	4	5.2	4.5	2.4
Speed skating	1 0	181	76.5	65 7	11 4
(n=6)	2.9	4	1 7	1 7	2.3
Long-distance runni g	.6.2	177	66.2	60.9	8 4
(n=8)	.8	4	3.2	3.0	1.5
Physical educatio students (=8)	25 6	178	71 7	62.8	11.0
	4.8	7	5 7	5.0	2.7
Control persons	30.3	176	75 0	61 6	14.4
(n=23)	6.5	7	11.8	8.4	3.0
Cross-country skiing	24.3	163	59 1	47 0	1 8
woman (n=5)	4 0	8	5.2	4.8	3 7

[a] Calculated as a mean of skinfold (Durnin and Rahaman 1967) and anthropometric (v Döbeln measurements.
[b] Acco di g to D rnl and Rahaman (1967).

decathlon 1). M ny of the athletes belonged to the Finnish national teams during the 1972 and 1974 and European championships and lympic games. All of the athletes have trained regularly for years, some of them more tha 10 years. The control persons (23) were policemen students and m of the niversity staff Most of them have exercised regularly according to their own fitness progr addition 8 male students of physical education were used as reference persons. T ble I summari physical characteristics of the subjects.

The followi g mai parameters were investigated on most of the subjects. vertical velocity arm f rces, blood lact te after maximal arm nd leg work, muscle fiber composition, activities of lact hydrogenase (LDH) and creatine phosphokinase (CPK) skeletal muscle tissue.

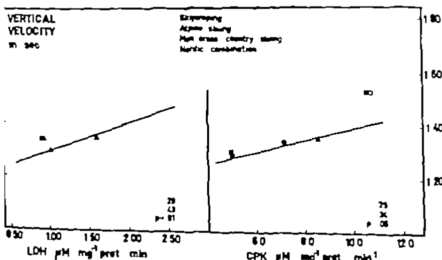
Vertical velocity was measured using the method of Margaria *et al* (1966) The subjects ra up th with maximal speed 2 steps t time. *The running velocity* was recorded electronically when the test speed was achieved after 4 steps. The recorded speed was converted to vertical velocity (V_v, m s muscular power (MP kgm s^{-1}). *Muscle force* of extensor muscles of both legs nd right elb measured isometrically using special dynamometers (Komi 1973 a, b). The angles of the knees a elbow were 107° and 94 respectively *Blood lactate* was measured from capillary blood taken fingertip 3 to 5 min after maximal treadmill runn g and maximal arm-ergometer test The reage instructions of Biochemica Boehringer ere used. *Muscle fiber composition and enzyme activiti*



Relationship between the activity of enzyme LDH and FT fiber distribution in the gastrocnemius and vastus lateralis (right) muscles for the various subject groups.

s of men and women cross-country skiers, nordic combination skiers, alpine skiers, ice y players, speed skaters and canoeists (range of means 31–42 %) were also significantly (p (0.01–0.05)) as compared to the control group.

activities of the enzymes LDH and CPK were highest among alpine skiers. In con- o this the endurance type athletes, cross-country and nordic combination skiers, had icantly lower mean activities for these enzymes (Table II).



3. Relationship between vertical velocity and the activities of enzymes LDH (left) and CPK (right) the various subject groups.

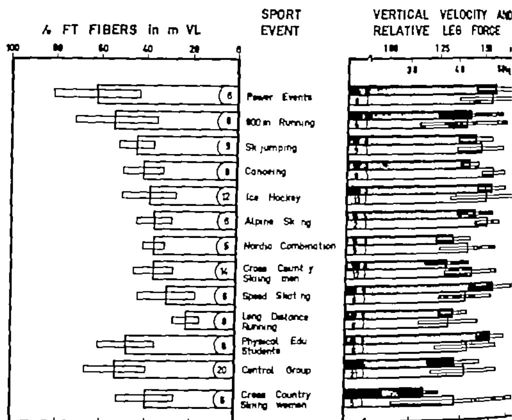


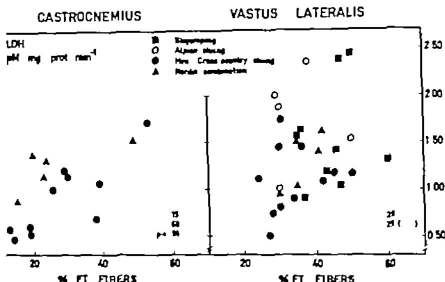
Fig. 1 Left Percent distribution of fast twitch fibers (% FT) in the vastus lateralis muscle of the d thlet and reference groups.
Right Vertical velocity (filled bars) and relative isometric leg force (open bars) for the same gro

athletes. Endurance athletes (skiers and runners) had lower vertical velocity as comp the other athlete groups and controls. Muscular power demonstrated a similar patte averaged in the athlete groups except for endurance athletes between 111 9 and 119 8 s whereas the male endurance athletes demonstrated values between 87 1 and 92.5 s which were below the values of controls The female cross-country skiers had 9 vertical velocity and 22% lower muscular power than their male counterparts.

Total and relative leg forces were recorded highest among power athletes, canoe hockey players, skijumpers and alpine skiers. Although the female cross-country ski 23% lower total and 8% lower relative leg forces than men skiers, their relative streng higher than that of the long distance runners.

Power athletes, 800 m runners, canoeists and alpine skiers had highest and almost (13.2–13 8 mM) peak blood lactate concentrations after the maximal treadmill ru speed skaters had the lowest mean value (9 0 ± 1 1 mM), which differed significant (0 01–0 05)) from the means of the other male athlete groups.

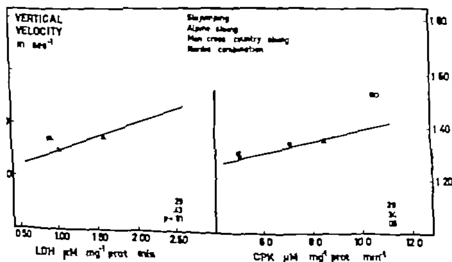
Muscle fiber distribution (Fig. 1) expressed as % FT fibers showed the highest mean of 63% in power athletes, although it did not differ significantly from the fiber distrib of the controls. In contrast to this the long-distance runners had, on the average, onl FT fibers in m. vastus lateralis (p < 0 001 as compared to th



Relationship between the activity of enzyme LDH and % FT fiber distribution in the gastrocnemius and vastus lateralis (right) muscles for the various subject groups

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…. 3. Relationship between vertical velocity and the activities of enzymes LDH (left) and CPK (right) … the various subject groups.

When the different variables were intercorrelated it was observed that total leg force was strongly related with body weight The relative force was therefore selected for further analysis. It correlated positively ($r = 0.38$, $p < 0.001$) with % FT fibers in m. VL of all male athletes (see also Fig. 1).

Vertical velocity correlated positively with the % FT fibers in male athletes ($r =$ [illegible], $p < 0.001$) (see also Fig. 1). In addition vertical velocity correlated ($p < 0.01$) with the relative leg force in this group With respect to the % FT fiber distribution only LDH activity of the enzymes studied was related as follows m. vastus lateralis $r = 0.29$ (p = n.s.) and gastrocnemius $r = 0.68$ ($p < 0.01$) (Fig. 2). Vertical velocity demonstrated significant relationships with the enzymes LDH and CPK in the male athletes (Fig. 3). These relationships were significant also in skijumpers ($r = 0.70$, $p < 0.05$ and $r = 0.75$ $p < 0.01$ respectively). In addition to these correlations peak blood lactate correlated significantly with the % FT fibers in the male athletes ($r = 0.24$ $p < 0.05$).

Discussion

In very short and intense events neuromuscular factors and energy utilization of the ATP and CP stores are essential. If the duration of effort is 1–4 min ATP resynthesis is derived also from the anaerobic glycolysis. In endurance sport events the anaerobic performance capacity has a smaller role and the energy is produced primarily through oxidative processes.

In the present study power athletes and skijumpers might be thought to represent a "phosphagen type" of sport event ice hockey players and alpine skiers primarily of anaerobic glycolysis type, and the other athletes in differing extents of aerobic type activities. However the variables of the study did not differentiate systematically the athlete groups on these three components. Although the measurements of muscular power and vertical velocity seemed to divide the athletes into anaerobic-neuromuscular and aerobic types, this statement cannot be generalized from the available data. It may be so that some specific needs of the different sport events mask the expected characteristics of energy and power utilization.

The potential value of the measurement of muscular power as a criterion of differentiation in the anaerobic power of individuals is justified and its use becomes more important when one considers that it in contrast to the maximal isometric force is the variable which is influenced primarily by the hereditary factors (Komi *et al* 1973 and 1976). Running velocity and muscular power seem also to be more clearly than muscle strength influenced by muscle tissue parameters such as activities of enzymes involved in ATP turnover and glucose residue metabolism Data for CPK and LDH from the present study and for CPK and MK from Komi *et al* (1976) strengthen this assumption.

The mean values of the % FT fiber distribution in VL muscle were different in the extreme athlete groups. In agreement with earlier reports (*e g* Gollnick *et al* 1972) long-distance runners had low percentage of FT fibers. The canoeists, alpinists, ice hockey players, speed skaters and skiers had similar mean % FT values but variances in the measurements allow athletes of differing structural potentialities to compete fairly succesfully in these sport events. Although the skijumpers as a group were relatively "slow" with respect to their % FT fiber distribution the inspection of the individual data revealed that the best

skijumper had the highest percentage of FT fibers from all measured skijumpers. That the fiber distribution may have some predictive value for the success in competition has been shown (Rusko *et al.* 1976).

The skijumpers had relatively low force values as compared to the endurance athletes or control persons. The close examination of the athletes yearly training diaries prior to testing revealed that the skijumpers were training unexpectedly heavily with endurance promoting exercises, which may take place at the expense or nonimprovement of some other functional components such as force (*e.g.* Gordon 1967).

Power athletes in general had relatively low % FT fiber distribution. Similar observations have been obtained in a Swedish sample of sprinters and jumpers of national caliber (Thorstensson, personal communication). Logically one would have expected that the top level power athletes have higher % FT value in their muscles. In fact two individual values for internationally succesful jumpers in track and field (unpublished data from our laboratory) and that for a superclass sprinter (Gollnick *et al.* 1972) suggest that power athletes of high international caliber should have % FT value of 70–90%.

Skeletal muscle fiber composition has been shown to be determined solely by heredity (Komi *et al.* 1976). Thus the top level athletes are, in addition to training, most likely products of strong genetic selection. This may be of special importance in sport events requiring specific components of neuromuscular and energy yielding processes. On national or lower level competitions athletes with weaker genetic potentials may however perform quite successfully. This is more likely in events, which cannot be characterized clearly enough with respect to neuromuscular and energy production criteria. The importance of hereditary factors are emphasized when one considers that the present state of knowledge objects the possibility of altering the skeletal muscle fiber composition through training. Endurance (Gollnick *et al.* 1973), sprint (Thorstensson *et al.* 1975) and strength training (Thorstensson *et al.* 1976) have all failed to show any change in % FT fiber distribution.

This study was supported by grants from the Ministry of Education, Finland and the Finnish Olympic Committee.

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Acta physiol. scand. 1977. 100. 115–121

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Differentiation of Vasoconstrictor Sensitivity Caused by Prostaglandin E in Peripheral Vascular Beds of the Rabbit

By

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Received 29 November 1976

Abstract

WENNMALM, Å. *Differentiation of vasoconstrictor sensitivity caused by prostaglandin E in peripheral vascular beds of the rabbit.* Acta physiol. scand. 1977. *100.* 115–121.

The increase in perfusion pressure in the rabbit ear, hindleg and mesentery caused by close intra-arterial injection of noradrenaline (NA), and the contractile response to NA of the rabbit aortic strip, are investigated with respect to their sensitivity to prostaglandin E₁ (PGE₁) and to the prostaglandin synthesis inhibitor indomethacin. PGE₁ (100–200 ng) potentiated the increases in perfusion pressure caused by NA in the perfused hindleg and mesentery, and the contractile response to NA of the aortic strip by 25–80 %, but inhibited the increase in perfusion pressure by NA in the perfused ear by 33–100 %. Indomethacin (3–5 · 10⁻⁶ M) significantly decreased the pressor responses to NA in the hindleg (by 45 %) and mesentery (by 35 %). This inhibitory effect by indomethacin was completely reversed by PGE₁. The responses to NA in the aortic strip and the perfused ear were unaffected by indomethacin. It is concluded that the process of vasoconstriction in the vascular beds of the rabbit displays qualitative differences concerning its sensitivity to added PGE₁. Furthermore, the decreased pressor responses to NA observed in some of the rabbit vascular beds after indomethacin indicate that the sensitivity to NA in these tissues in fact is increased by endogenous prostaglandin-like substances (PLS). The current results thus suggest that endogenous PLS may regulate, at a local level, the vasoconstrictor sensitivity in the rabbit systemic resistance vessels.

Prostaglandins of the E series may influence the regulation of the blood circulation by increasing or decreasing the vasoconstrictor response to sympathetic stimuli. Thus, exogenous PGE have been reported to act as either facilitators or inhibitors—depending on the tissue and species investigated—on the vasoconstrictor response to adrenaline or noradrenaline in various blood vessels (Kadowitz 1972, Greenberg and Long 1973, Zimmerman *et al.* 1973, Horrobin *et al.* 1974).

Furthermore, inhibition of the synthesis of prostaglandin-like substances in the rabbit with indomethacin has been reported to cause an increase in systemic blood pressure (Lonigro *et al.* 1973, Larsson and Änggård 1973). This may indicate that endogenous PLS influence the blood circulation, by maintaining a slight degree of vasodilation in the systemic vascular bed in this species. Such a dilatory action may well be the final result of different or even

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...constant pressure responses to NA were obtained, the responses to the combined injection of the same amount of NA and PGE (100 ng) were recorded. Subsequently control injections of NA only were given. The series of injections was then repeated after the addition of indomethacin (3–5 10^{-6} M) to the Tyrode solution perfusing the hindleg.

) Mesenteral artery

The abdominal cavity of the rabbit was opened by midline incision and the superior mesenteric artery was dissected free and cannulated distally. The mesenteric vascular bed was flushed with saline, which was allowed to drain via an incision in the portal vein. The system was then perfused at a flow rate of 3 ml/min, which produced a pressure of 20–40 mmHg. The equipment for pressure recording and perfusion was the same as mentioned above. After 30 min equilibrium period the pressure responses to injections of NA 1 2 µg) in the absence and in the presence of PGE (100 ng) were assayed. The pressure responses to NA, and NA PGE, were also assayed after addition of indomethacin (3–5 10^{-6} M) to the Tyrode solution perfusing the vascular bed.

Calculations

The effect of PGE_1 on the pressure responses to NA in the different vascular beds perfused was estimated as the ratio between the first pressure response to NA in the presence of PGE and the preceding pressure response to NA only. The effect of indomethacin on the pressure response to NA was estimated similarly. The values for the effect of PGE_1 or indomethacin on the pressure responses to NA are presented as mean S.E. (standard error of the mean). Figures within brackets indicate number of experiments. Student's t-test has been used (paired means) when applicable.

Results

A. *Aortic strip* (Fig. 1 Table I)

The basal activity of the aortic strip was very low and the base line was stable. Addition of NA (50–200 ng) to the bath elicited strong contractions of the organ. The contractions to repeated additions of the same dose of NA increased in the initial stages but remained constant after 3–4 additions of NA. When PGE_1 (100 ng 5 10^{-6} M) was added to the bath together with NA, the contractile response was potentiated by 80%. The potentiating effect of PGE on the contractile response of the strip to NA was unaffected by the addition of indomethacin (3–5 10^{-6} M) to the bath solution. Indomethacin, in the dose mentioned, did not *per se* affect the contractile response of the aortic strip to added NA.

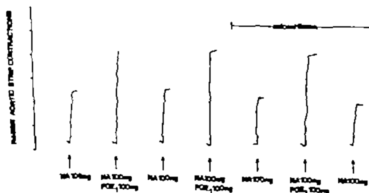


Fig. 1. Contractions of the helically cut strip from the rabbit thoracic aorta mounted in 5 ml organ bath and exposed during 90 to noradrenaline (NA, 100 ng) in the absence or presence of PGE_1 (100 ng). ● Indicates washing of the bath. Indomethacin (3 10^{-6} M) present in the perfusing medium as indicated.

opposite effects (*cf* above) elicited by endogenous PLS on the vascular reactivity in t various sections of the resistance bed If this is true, endogenous PLS, by inducing differ effects on the vascular reactivity in different tissues, may play an important role in t distribution of the blood flow Such an action by endogenous PLS would require, primari that the reactivity to NA in different parts of the resistance vasculature differs in its sen tivity to exogenous PGE, and secondarily that inhibition of the endogenous synthesis PLS is followed by a change in the vascular sensitivity to NA (caused by the elimination endogenous PLS).

In the current investigation these two hypotheses have been tested in some isolated par of the rabbit vascular bed, chosen to represent skeletal muscle, skin, mesentery and cere systemic vessels.

Methods

Rabbits of either sex, weighing 1 5–3.2 kg, were used. They were stunned by a blow on the head and e sanguinated via the left carotid artery Subsequently some of the following preparations were perform

A *Aortic strip*

A 20 mm section of the thoracic aorta was removed and cut helically to produce a strip measuring appro mately 4 50 mm. This was mounted in an organ bath containing 4.5 ml of +37°C Tyrode solution of the followi g composition in mM Na 149 K 2.7 Ca^{++} 1.8, Mg^{++} 1.0, Cl^- 145 HCO_3 0.4, glucose 11 The solution was continuously aerated with 5% CO in O The lower end of the strip was mounted in the bottom of the organ bath and the upper end was connected to an isotonic Harvard Heart/Smooth Mus Transducer The isotonic co tractions of the aortic strip were recorded via the transducer on a Watanab I k Writer After a 30 min equilibration period in the organ bath, the contractions of the strip hen expos during 90 s to NA (50–200 ng of NA added to the bath) were assayed. When reproducible contractions NA were obtained the effect of PGE_1 (5 10^{-8} M) on the sensitivity of the strip to NA as assayed b simultaneous addition of these drugs to the bath The sensitivity of the strip to NA i the absence and the presence of PGE was also tested fter addition of indomethacin (3–5 10^{-6} M) to the Tyrode solution

B *Ear artery*

The ear was removed nd mounted vertically with its cranial end upwards in a wide beaker contain +30°C water The central artery of the ear was can ulated in distal direction by a 0.5/0.8 mm polyethylen tube connected t 100 ml syringe in a Sage 351 i fusion pump. The cannulation point of the artery w adjusted t be 10 mm higher tha the upper edge of the beaker The marginal vein effluent from the e was allowed to drip freely into the beaker The pressure on the perfusing side of the system as measu as side pressure via a side branch of the polyethylene tubing ttached to an Elema EMT 34 pressure tra ducer The transducer was connected to n Elema EMT 31 amplifier and the pressure as monitored o the Watanabe Ink Writer The flow usually 3 ml/min, was chosen t produce a mean perfusion pressu of 40–50 mmHg. Repeated pressure calibrations, using electrical stand rd, were performed. After a 30-4 mi equilibration period, the pressure responses t repeated injections of NA (1 μg, administered v side branch i the polyethylene tubing) were tested. When reproducible pressure responses to NA we obtained, the effect of simultaneous injections of PGE_1 (100–200 ng) and NA on the pressure response t NA were recorded. The procedure was subsequently repeated in the presence of indomethacin (3 5 10^{-6} M in the Tyrode solution perfusi g the ear

C *Femoral artery*

A short incision was made in either of the inguinal regions of the rabbit and the femoral artery and vein we dissected free. The artery was cannulated in distal direction. The femoral vein was divided. After flushi of the hindleg vascular bed with saline via the arterial cannula, the cannula was connected to the syrin pump mentioned above. The hindleg was perfused at a flow rat of 3 ml/min, which produced a pressu of 15–30 mmHg. The recording equipment was the same as described bove. After a 30–45 min equilibratio period the increase in perfusion pressure caused by single injections of NA (1 2 μg) was recorded. Whe

constant pressure responses to NA were obtained, the responses to the combined injection of the same amount of NA and PGE_1 (100 ng) were recorded. Subsequently control injections of NA only were given. The series of injections was then repeated after the addition of indomethacin (3 5 10^{-6} M) to the Tyrode solution perfusing the hindleg.

Mesenterial artery

The abdominal cavity of the rabbit was opened by a midline incision and the superior mesenteric artery was dissected free and cannulated distally. The mesenteric vascular bed was flushed with saline, which was allowed to drain via an incision in the portal vein. The system was then perfused at a flow rate of 3 ml/min, which produced a pressure of 20–40 mmHg. The equipment for pressure recording and perfusion was the same as mentioned above. After a 30 min equilibrium period the pressure responses to injections of NA (2 µg) in the absence and in the presence of PGE_1 (100 ng) were assayed. The pressure responses to NA, and NA + PGE_1 were also assayed after addition of indomethacin (3–5 10^{-6} M) to the Tyrode solution perfusing the vascular bed.

Calculations

The effect of PGE_1 on the pressure responses to NA in the different vascular beds perfused was estimated as the ratio between the first pressure response to NA in the presence of PGE_1 and the preceding pressure response to NA only. The effect of indomethacin on the pressure response to NA was estimated similarly. The values for the effect of PGE_1 or indomethacin on the pressure responses to NA are presented as mean ± S.E. (standard error of the mean). Figures within brackets indicate number of experiments. Student's t-test has been used (paired values) when applicable.

Results

A. *Aortic strip* (Fig. 1, Table I)

The basal activity of the aortic strip was very low and the base line was stable. Addition of NA (50–200 ng) to the bath elicited strong contractions of the organ. The contractions to repeated additions of the same dose of NA increased in the initial stages but remained constant after 3–4 additions of NA. When PGE_1 (100 ng 5 10^{-6} M) was added to the bath together with NA, the contractile response was potentiated by 80 %. The potentiating effect of PGE_1 on the contractile response of the strip to NA was unaffected by the addition of indomethacin (3–5 10^{-6} M) to the bath solution. Indomethacin, in the dose mentioned, did not *per se* affect the contractile response of the aortic strip to added NA.

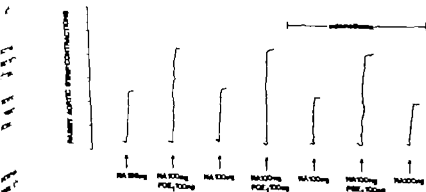


Fig. 1. Contractions of the helically cut strip from the rabbit thoracic aorta mounted in a 5 ml organ bath and exposed during 90 s to noradrenaline (NA, 100 ng) in the absence or presence of PGE_1 (100 ng). ● indicates rinsing of the bath. Indomethacin (3 10^{-6} M) present in the perfusing medium as indicated.

opposite effects (*cf* above) elicited by endogenous PLS on the vascular reactivity in the various sections of the resistance bed. If this is true, endogenous PLS, by inducing different effects on the vascular reactivity in different tissues, may play an important role in the distribution of the blood flow. Such an action by endogenous PLS would require, primarily, that the reactivity to NA in different parts of the resistance vasculature differs in its sensitivity to exogenous PGE, and secondarily that inhibition of the endogenous synthesis of PLS is followed by a change in the vascular sensitivity to NA (caused by the elimination of endogenous PLS).

In the current investigation these two hypotheses have been tested in some isolated parts of the rabbit vascular bed, chosen to represent skeletal muscle, skin, mesentery and central systemic vessels.

Methods

Rabbits of either sex, weighing 1.5–3.2 kg, were used. They were stunned by a blow on the head and exsanguinated via the left carotid artery. Subsequently some of the following preparations were performed.

A. *Aortic strip*

A 20 mm section of the thoracic aorta was removed and cut helically to produce a strip measuring approximately 4 × 50 mm. This was mounted in an organ bath containing 4.5 ml of +37°C Tyrode solution of the following composition in mM: Na^+ 149, K^+ 2.7, Ca^{++} 1.8, Mg^{++} 1.0, Cl^- 145, HCO_3^- 0.4, glucose 5.5. The solution was continuously aerated with 5% CO_2 in O_2. The lower end of the strip was mounted in the bottom of the organ bath and the upper end was connected to an isotonic Harvard Heart/Smooth Muscle Transducer. The isotonic contractions of the aortic strip were recorded via the transducer on a Watanabe Ink Writer. After a 30 min equilibration period in the organ bath, the contractions of the strip when exposed during 90 s to NA (50–700 ng of NA added to the bath) were assayed. When reproducible contractions to NA were obtained, the effect of PGE_1 (5 × 10^{-8} M) on the sensitivity of the strip to NA was assayed by simultaneous addition of these drugs to the bath. The sensitivity of the strip to NA in the absence and the presence of PGE was also tested after addition of indomethacin (3–5 × 10^{-6} M) to the Tyrode solution.

B. *Ear artery*

The ear was removed and mounted vertically with its cranial end upwards in a wide beaker containing +30°C water. The central artery of the ear was cannulated in distal direction by a 0.5/0.8 mm polyethylene tube connected to a 100 ml syringe in a Sage 351 infusion pump. The cannulation point of the artery was adjusted to be 10 mm higher than the upper edge of the beaker. The marginal vein effluent from the ear was allowed to drip freely into the beaker. The pressure on the perfusing side of the system was measured as side pressure via a side branch of the polyethylene tubing attached to an Elema EMT 34 pressure transducer. The transducer was connected to an Elema EMT 31 amplifier and the pressure was monitored on the Watanabe Ink Writer. The flow, usually 2–3 ml/min, was chosen to produce a mean perfusion pressure of 40–50 mmHg. Repeated pressure calibrations, using electrical standard, were performed. After a 30 min equilibration period, the pressure responses to repeated injections of NA (1–2 µg, administered in a side branch in the polyethylene tubing) were tested. When reproducible pressure responses to NA were obtained, the effect of simultaneous injections of PGE_1 (100–200 ng) and NA on the pressure responses to NA were recorded. The procedure was subsequently repeated in the presence of indomethacin (3–5 × 10^{-6} M) in the Tyrode solution perfusing the ear.

C. *Femoral artery*

A short incision was made in either of the inguinal regions of the rabbit and the femoral artery and vein were dissected free. The artery was cannulated in distal direction. The femoral vein was divided. After flushing of the hindleg vascular bed with saline via the arterial cannula, the cannula was connected to the syringe pump mentioned above. The hindleg was perfused at a flow rate of 3 ml/min, which produced a pressure of 15–30 mmHg. The recording equipment was the same as described above. After a 30–45 min equilibration period the increase in perfusion pressure caused by single injections of NA (1–2 µg) was recorded. When

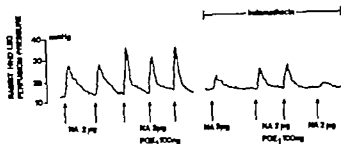


Fig. 3. Perfusion pressure in the rabbit femoral artery during repeated injections of noradrenaline (NA, μg) in the absence (inj. 1–2, 6, 9) or in the presence (inj. 3–5, 7–8) of PGE_1 (100 ng). Indomethacin (5 · 10^{-6} M) present in the perfusing medium as indicated.

Indomethacin (3–5 · 10^{-6} M) added to the medium perfusing the ear artery to some extent affected the responses to added NA, inasmuch as the effect of PGE on the pressure responses to NA was then always inhibitory. In most experiments, NA added together with PGE failed to elicit any pressure response after the addition of indomethacin to the medium.

C. *Femoral artery* (Fig. 3, Table I)

At a perfusion flow of 3 ml/min, the pressure of the perfused hindleg of the rabbit was 15–30 mmHg. Spontaneous fluctuations of the perfusion pressure were observed, in the range 2–4 mmHg, but the basal pressure was always constant. Addition of NA (2 μg) caused an increase in the perfusion pressure, in the range 10–30 mmHg. The pressure responses to injections of NA became constant after 2–3 NA administrations. PGE_1 elicited an insignificant increase (by 25%) in the pressure response of the hindleg to NA. Repeated simultaneous administrations of NA and PGE_1 elicited reproducible pressure responses, and in no experiment was the facilitatory effect of PGE on the pressure response to NA converted into inhibition.

Addition of indomethacin (3–5 · 10^{-6} M) to the medium perfusing the hindleg significantly decreased the pressure response to NA to about 55 % of the controls. Repeated injections of NA elicited reproducible pressure responses, but always at a lower level than before the administration of indomethacin. In some experiments subsequent perfusion of the hindleg with indomethacin-free Tyrode solution was performed. In these experiments the pressure responses to added NA gradually returned towards the level observed before the administration of indomethacin. Thus, the depressing effect of indomethacin on the pressure responses of the hindleg to NA was reversible. PGE_1 completely counteracted the depressing effect by indomethacin on the pressure responses to NA.

D. *Mesenterial artery* (Fig. 4, Table I)

The perfusion pressure in the mesenteric vascular bed was 20–40 mmHg at constant flow of 3 ml/min. Small variations (2–3 mmHg) were observed but the basal pressure remained constant. Addition of NA (1–2 μg) elicited, within 3–4 additions, constant pressure responses, amounting to 15–25 mmHg. These pressure responses were potentiated when PGE (100 ng) was added together with the NA. Repeated simultaneous injections of NA and PGE caused

TABLE I Effect of PGE_1—in the absence and in the presence of indomethacin— and of indomethacin alone, on the mechanical responses to NA in different parts of the rabbit systemic vascular bed. The effects are expressed as the ratio between the respone to NA after and before the addition of PGE_1 or indomethacin. The figures presented indicate mean ±S.E., except for the effects of PGE_1 on the responses to NA in the ear artery where—due to the qualitative differences in responses (cf. "Results")—the range is given. Figures in brackets indicate number of experiments. * indicates that the ratio differs significantly (P<0.01) from 1.00. ‡ indicates that the value differs significantly (P<0.02) from the corresponding figure before indomethacin.

	$\frac{NA+PGE}{NA}$ Before indomethacin	$\frac{NA+PGE}{NA}$ After indomethacin	$\frac{\text{NA after indomethacin}}{\text{NA before indomethacin}}$
Aortic strip (6)	1.79±0.18	1.06±0.19	0.97±0.09
Ear artery (5)	1.45–0.00	0.89–0.00	0.85±0.16
Femoral artery (9)	1.25±0.16	2.23±0.44 ‡	0.53±0.09
Mesenterial artery (5)	1.39±0.19	3.39±0.72 ‡	0.47±0.08

B *Ear artery* (Fig. 2, Table I)

The perfusion flow applied produced a pressure of 40–50 mmHg in ear which was maintained during the entire experiment. The recordings revealed regular minor spontaneous variations in the pressure, in the range 2–3 mmHg. Addition of NA (1–2 µg) to the perfusing medium elicited a rapid, brief increase in the perfusion pressure. The increase ranged between 20 and 60 mmHg and lasted not more than 1 min. After 2–3 additions of NA the responses to subsequent additions were identical. Simultaneous addition of NA (1–2 µg) and PGE (100–200 ng) in most cases elicited a smaller pressure response than did NA alone in the range 0 to 65% of the control. In some experiments, however, an initial potentiating effect of PGE was obtained on the pressure response to added NA. During repeated simultaneous additions of NA and PGE this potentiation maximal 45 % was rapidly converted into an inhibition and consequently the overall final effect of PGE_1 on the pressure response by the ear artery to NA was a definite, often total, inhibition.

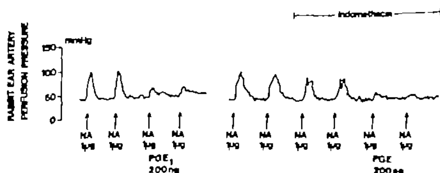


Fig. 2. Perfusion pressure in the rabbit ear artery during repeated injections of noradrenaline (NA, 1 µg) in the absence (inj. 1–2, 5–6) or in the presence (inj. 3–4, 9–10) of PGE (200 ng). Indomethacin (3 × 10⁻⁵ M) present in the perfusing medium as indicated.

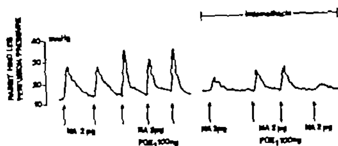


Fig. 3. Perfusion pressure in the rabbit femoral artery during repeated injections of noradrenaline (NA, μg) in the absence (inj. 1, 2, 6, 9) or in the presence (inj. 3–5, 7–8) of PGE (100 ng). Indomethacin (5 10^{-5} M) present in the perfusing medium as indicated.

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Femoral artery (Fig. 3, Table I)

At a perfusion flow of 3 ml/min, the pressure of the perfused hindleg of the rabbit was 15–30 mmHg. Spontaneous fluctuations of the perfusion pressure were observed, in the range –4 mmHg, but the basal pressure was always constant. Addition of NA (μg) caused an increase in the perfusion pressure, in the range 10–30 mmHg. The pressure responses to injections of NA became constant after 2 3 NA administrations. PGE elicited an insignificant increase (by ∠5%) in the pressure response of the hindleg to NA. Repeated simultaneous administrations of NA and PGE_1 elicited reproducible pressure responses, and in no experiment was the facilitatory effect of PGE on the pressure response to NA converted into inhibition.

Addition of indomethacin (3–5 10^{-5} M) to the medium perfusing the hindleg significantly decreased the pressure response to NA to about 55% of the controls. Repeated injections of NA elicited reproducible pressure responses, but always at a lower level than before the administration of indomethacin. In some experiments subsequent perfusion of the hindleg with indomethacin-free Tyrode solution was performed. In these experiments the pressure responses to added NA gradually returned towards the level observed before the administration of indomethacin. Thus, the depressing effect of indomethacin on the pressure responses of the hindleg to NA was reversible. PGE_1 completely counteracted the depressing effect by indomethacin on the pressure responses to NA.

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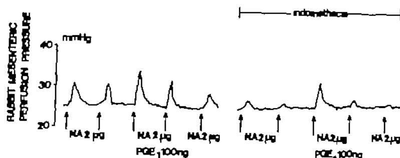


Fig 4 Perfusion pressure in the rabbit mesenterial artery during repeated injections of noradrenaline (NA, µg) in the absence (inj. 1 2, 5–7 10) and in the presence (inj 3–4 8–9) of PGE$_1$ (100 ng) Indomethacin (4×10^{-4} M) present in the perfusing medium s indicated.

constant pressure responses, and no fading of the potentiating effect of PGE was observed. Indomethacin significantly reduced the pressure responses to NA, to about 45 % of controls. As long as indomethacin was present in the Tyrode solution, the pressure responses to NA remained at this depressed level When the system was perfused with drug-free solution, the pressure responses to NA gradually returned to the original level. The inhibitory effect of indomethacin on the pressure responses to NA was completely reversed by PGE$_1$, in analogy with the observations in the hindleg.

Discussion

In the present study PGE was found to augment the vasoconstriction caused by NA in the rabbit hindleg and mesenteric vascular bed and to facilitate the contractions to NA of the aortic strip from this species. Furthermore, PGE was found to decrease the vasoconstriction caused by injection of NA in the ear artery This demonstrates that PGE influences the mechanical responses caused by NA, in different or even opposite ways in the various vascular beds of the rabbit It has earlier been shown that PGs influence the action of pressor agents in various preparations in a facilitatory as well as an inhibitory direction (for references see Introduction") but in those studies not only differences in the vascular beds investigated, but also the species and types of PGs used, may have contributed to the divergent results. In the present investigation those possibilities did not exist since only one species and one type of PG (PGE) were used Furthermore, in many cases vascular beds from the same animal were investigated, which further stresses the significance of the differences observed It should be noted that PGE constantly interacted with the pressor responses to NA in a facilitatory or inhibitory direction without affecting the basal pressure level. This demonstrates that the dose of PGE given together with NA was too small to affect the basal tone of the smooth muscle in the vessel walls directly but sufficient to affect its sensitivity to NA.

Indomethacin is an inhibitor of the synthesis of PLS as shown by Vane (1971) This drug decreased the responses to NA of the hindleg and mesenteric vascular beds by about 50 and left the responses to NA in the ear artery and the aortic strip unaffected. The effect of indomethacin was probably not the result of a direct action of the drug on the vascular sensitivity to NA, since equimolar drug concentrations would hardly elicit different effects

different vascular beds. It rather appears that indomethacin acted indirectly via inhibition the local formation of PLS. This assumption is supported by the fact that PGE_1 given gether with NA, completely restored the pressor responses in the hindleg and mesentery consequence of this observation is that in the hindleg and mesenteric ascular beds, endogenous PLS normally (in the absence of indomethacin) increase the smooth muscle sensitivity to NA. The lack of effect of indomethacin on the responses to NA in the ear artery d the aortic strip suggests, according to the same theory that no endogenous formation of PLS capable of affecting vascular reactivity was going on in these tissues. On the sis of the indomethacin effects observed in the different preparations, it is therefore suggested that locally formed PLS affect the smooth muscle sensitivity to NA differently in fferent regions of the systemic vascular bed. Results suggesting that an endogenous PLS rmation is necessary for the "normal" activation of the rat mesenteric artery adrenoceptors ve been reported earlier (Horrobin *et al* 1974). The current results extend these observations to another species and to an additional vascular bed, namely that of the hindleg in the rabbit Furthermore, the results demonstrate that decreased vascular sensitivity to NA following administration of a PLS synthesis inhibitor occurs in some, but not all vascular eds.

Summing up, the results demonstrate the existence of important qualitative differences etween some ascular beds of the rabbit in their smooth muscle sensitivity to PGE These fferences may constitute physiological basis for the regulation of the resistance vascular ed sensitivity to NA, elicited by locally produced PLS

This study as supported by the Swedish Medical Research Council, project 04X–4341 and by Magnus ergvalls stiftelse

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Acta physiol. scand 1977 100. 122-125

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The Poor Reactivity of Retinal Vessels to Systemic Administration of Vasoactive Agents in Pentobarbital Anesthetized Rats

By

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Received 1 December 1976

Abstract

YLITALO P., P ERTAMA and L. ERTAMA. *The poor reactivity of retinal vessels to syst administration of vasoactive agents in pentobarbital anesthetized rats.* Acta phy scand. 1977 *100* 122-125

The responsiveness of retinal vasculature to i. adm nistratio of several potent vasoactive agents studied pentobarbital anesthetized rats by taking f dus photographs. Since cerebral asculatur been claimed to react in a similar manner but less liably than retinal vessels to some vasoactive substa the f nd ngs were pplied t the problem of reactivity of brain vessels. Sublethal doses of noradrenc adrenal ne, 5-hydro ytrypta nine ngiotensin amide d argi ine or lysine vasopressin caused no m acute (2 mi) vasoconstricti in retinal vessels. N did any of these agents or bradykinin elicit dilat tio The late vasoco striction (2 min) found i succumbing animals was most likely unspe since it did not occur unt l severe to ic symptoms appeared The findings support the concept that cerebral vessels are q te res stant t the direct act on of ma y vasoactive agents given i

Key words. Vasoactive agents, retinal vessels, cerebral vessels, nesthetized rats

The retinal vasculature has been claimed to react in a similar manner but more suscep to vasoactive agents than cerebral vessels (Bailliart 1941 Bailliart *et al* 1956 a and 195 which are reported to be quite resistant to some vasoactive substances (Bovet *et al* 1 Bovet and Carpi 1958 Sautter *et al* 1954 Swank and Hissen 1964). Changes in the cal of retinal vessels can be easily estimated also in small laboratory animals without opera or other crude procedures (Ertama and Ylitalo 1972, Tammisto 1965) which may mo the responsiveness of blood vessels. In this respect, we have studied the responsivenes retinal vasculature to systemic administration of many potent vasoactive agents in a thetized rats by taking photographs of the fundus, and findings were also applied to problem of reactivity of cerebral vessels.

Material and methods

Male Sprague–Dawley rats weighing 100–150 g were fed with standard laboratory pellets and tap water *ad libitum*. The test-room temperature was 20–23°C.

Rats were anesthetized with 40 mg/kg of pentobarbital sodium (Star Ltd., Tampere) i.p. 20 min before injecting the following vasoactive agents into tail vein in a volume of 2 ml/kg of b.w.: noradrenaline 20 µg/kg (noradrenaline bitartrate, Nor-Adrenalin, Star Ltd., Tampere), adrenaline 50 µg/kg (Adrenalin, Medica Ltd., Helsinki), 5-hydroxytryptamine 50 and 100 µg/kg (5-hydroxytryptamine creatinine sulphate, 5HT, Fluka AG, Buchs SG), angiotensin II amide 5 and 25 µg/kg (Hypertensin, Ciba-Geigy, Basle), arginine vasopressin 5 IU/kg (Ferring Ab, Malmö), lysine vasopressin 5 IU/kg (Ferring Ab, Malmö) and bradykinin 0.4 mg/kg (Nutritional Biochemicals Corp., Cleveland, Ohio).

For the photography of retinal vessels pupillary dilatation was caused by one drop of 1% tropicamide on the cornea. The retinal vessels were photographed with a Zeiss fundus camera (Opton 1117) equipped with a green filter (Wratten 58). The calibres of retinal vessels were estimated by measuring their diameter at 25-fold magnification of negatives which were projected onto white paper (Laatikainen 1971). The significance of changes in calibre was tested by the t-test for paired observations.

Results and discussion

No marked acute (< 2 min) vasoconstriction was found in retinal vessels of any rats after the administration of sublethal doses of noradrenaline, adrenaline, 5HT, angiotensin amide and arginine or lysine vasopressin (Fig. 1). Neither did any significant increase occur in the diameter of retinal arteries or veins after the administration of bradykinin or other agents some of which also have vasodilatatory properties, but in a few rats retinal veins tended to be slightly dilated.

With the exception of a short apnea, the surviving animals (about 30 %) usually showed no other toxic symptoms, and retinal vessels remained unconstricted also later on, i.e. through the observation period of 15 min. In succumbing rats (about 70 %), however, a significant vasoconstriction (p < 0.05) was found at 2 to 3 min after the administration of vasoactive agents. These animals had a more pronounced primary apnea and subsequently signs of severe hypoxia, e.g. cyanosis and scratching. No reactive vasodilatation could be observed in them, and thereafter, regardless of excellent focusing of the camera, the retinal vessels could no longer be visualized accurately, since the optical pathway became cloudy. On very rare occasions the animals with severe hypoxia survived, and in such a case the vasoconstriction and the cyanosis was found to disappear when the ventilation was normalized. Because the vasoconstriction in retinal vessels did not occur until pronounced toxic symptoms appeared, the late constriction had to be secondary and thus most likely unspecific in nature.

Although barbiturates interfere with the nervous control of the circulation (Price 1960, Strobel and Wollman 1969), the response of blood vessels to vasoactive compounds is well maintained under these experimental conditions. This is uniformly documented in many previous studies in which the relative small and nontoxic doses of vasoconstrictive agents have been shown to cause a considerable increase in the blood pressure of pentobarbital anesthetized animals (Morris *et al.* 1950, Price 1960, Ylitalo 1972).

In the present study systemic i.v. injection of vasoactive agents in sublethal doses caused primarily no marked changes in the diameter of retinal vessels in pentobarbital anesthetized rats. Since cerebral vasculature has been claimed to be even less reactive than retinal vessels

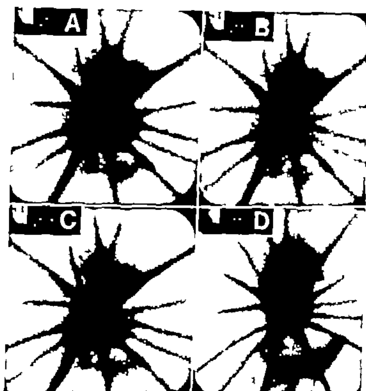


Fig. 1 A typical illustration of the nfluence of asoactl e gents on the retinal vessels tested in pentobarbital anesthetized rat. This f gure represents the effect of ngiotensin mide. A Before the drug, B after the drug C 100 s fter the drug; D The late vasoconstriction duri g the severe hypoxia just prior to cardiac arrest.

to some of these substances (Sautter *et al* 1954), the preceding finding support the conc that intracerebral vessels are quite resistant to systemic administration of many vasoact agents.

W are grateful to Profeso Salme Va nas, M D f the opportunity to tak fundus photographs of eyes, and t Mr Erkki M nn nen who very sk lf lly photographed them.

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Acta physiol. scand. 1977 100, 126–128

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Sustained Epileptic Seizures Complicated by Hypoxia, Arterial Hypotension or Hyperthermia. Effects on Celebral Energy State

By

G Blennow B Nilsson and B K Siesjö

Although it has been established that sustained epileptic seizures in animals are accompanied by permanent neuronal damage the causative mechanisms are unknown. Working with sustained seizures in baboons induced by bicuculline, pentylenetetrazole or allylglycine, Meldrum *et al* (1973 1975) speculated whether the histopathological damage was due to complicating factors such as hypotension, hypoxia, hyperthermia or hypoglycemia, or could be attributed to injurious effects of the epileptic activity as such.

Recent experiments in artificially ventilated rats have shown that sustained epileptic seizures induced by *i.v.* bicuculline are accompanied by a 2 to 3-fold increase in cerebral metabolic rate (Borgström *et al* 1976, Meldrum and Nilsson 1976). However although there was a relatively marked lactic acidosis, cerebral energy state as evaluated from the tissue concentrations of PCr ATP ADP and AMP were only moderately changed (Chapman *et al* 1977) In spite of this, and of the fact that there was no reductions in arterial P_{O_2} blood pressure or blood glucose concentration, or increase in body temperature, moderate neuronal damage is observed after 2 h of seizure activity (Blennow *et al* in preparation).

In the present expts. we have induced epileptic activity for 2 h by means of bicuculline in animals that were deliberately made hypoxic, hypotensive or hyperthermic. Labile cerebral metabolites were assessed at the end of the seizure period with the objective of studying whether or not these complicating factors may exaggerate the effect of seizures on cerebral energy state

Unstarved male Wistar rats (3.5–385 g) were anesthetized, immobilized and artificially ventilated on 70% N_2O with control of blood pressure, arterial blood gases, pH temperature and EEG Sustained seizures were induced for 2 h by *i.v.* injection of bicuculline (5 gms) a dose of 1.2 mg kg⁻ (see Chapman *et al* 1977). There were 4 groups. In one, measures were taken to alter brain oxygen supply or body temperature In the second group, mean arterial blood pressure was reduced to and maintained at 75 mmHg by arterial bleeding. In the third group arterial P_{O_2} was reduced to about 50 mmHg by lowering the O_2 concentration in the gas mixture, and the fourth group body temperature was elevated to about 40 C by external heating. These changes were induced after 5 min of seizure activity and the desired values for P_{O_2} and blood pressure were obtained after maximally 20 min, while that for body temperature was obtained after 28–40 min After 2 h of seizure activity the brain was frozen *in situ* for subsequent measurements of labile cerebral metabolites (see Chapman *et al.* 1977).

Table 1. Labile cerebral metabolites in rat cerebral cortex after 2 h of sustained bicuculline-induced epileptic seizures in control animals (normoxic, normotensive, normothermic) and in animals rendered hypotensive (mean arterial blood pressure 75 mmHg), hypoxic (P_{aO_2} 44.2 ± 3.5 mmHg), or hyperthermic (body temperature 40.3 ± 0.1°C). Means ± S.E. in μmol g^{-1} of wet tissue. * p 0.05, ** p 0.01, *** p 0.001

	Control N 5	Hypotension N 6	Hypoxia N 6	Hyperthermia N 5
Phosphocreatine	3.39 ± 0.27	2.65 ± 0.10*	2.61 ± 0.30	2.38 ± 0.13**
Creatine	6.34 ± 0.37	7.51 ± 0.76	7.55 ± 0.34	7.70 ± 0.20*
ATP	2.94 + 0.03	2.94 ± 0.04	2.80 ± 0.06	2.93 ± 0.03
ADP	0.324 ± 0.012	0.339 ± 0.008	0.340 ± 0.020	0.345 ± 0.012
AMP	0.048 ± 0.002	0.053 + 0.002	0.049 ± 0.002	0.051 ± 0.002
EC	0.937 ± 0.002	0.933 ± 0.002	0.931 ± 0.004	0.933 ± 0.002
Lactate	9.14 + 1.76	16.06 ± 1.95	23.22 ± 2.03***	17.72 ± 1.55
Pyruvate	0.166 ± 0.016	0.174 ± 0.008	0.171 ± 0.012	0.186 ± 0.012
L/Py	52.64 ± 7.48	92.53 ± 4.78*	143.88 + 23.10**	95.03 ± 13.28
NH_4	0.579 ± 0.025	0.664 ± 0.089	1.188 + 0.173**	0.747 ± 0.068**

EC adenylate "energy charge" $\frac{[ATP] + 0.5[ADP]}{[ATP] + [ADP] + [AMP]}$

In the control group (uncomplicated seizures) body temperature at the end of the 2 h period was 37.2 ± 0.2°C (mean ± S.E.). Mean arterial blood pressure (MABP) was 122 ± 5 mmHg, while arterial P_{aO_2}, P_{aCO_2} and pH were 108 ± 5 mmHg, 36.3 ± 1.7 mmHg and 7.165 + 0.025, respectively. In the hypotensive group, mean arterial blood pressure was 75 mmHg. In the hypoxic group arterial P_{aO_2} was 44.2 ± 3.5 mmHg and MABP was kept within 100–125 mmHg (2 animals had short-lasting transient falls down to 50 mmHg). In the hyperthermic group, finally, body temperature was 40.3 ± 0.1°C. In none of these last 3 groups did the EEG pattern deviate from that recorded in the control animals (uncomplicated seizures). All values given above are mean ± S.E.

Table 1 shows the labile cerebral metabolites. In the control group there were moderate changes in PCr and adenylate energy charge, increases in lactate concentration and lactate/pyruvate ratio, and a rise in ammonia concentration as compared to values obtained in animals without seizures. These changes are in agreement with previous results (*Chapman et al. 1977*). Except for a lower glycogen concentration in the hypoxic and hyperthermic animals, the values for glucose and glycogen were similar in all 4 groups (not shown). There were changes in phosphocreatine and/or creatine concentrations in the hypotensive, hypoxic and hyperthermic groups, and all showed increases in lactate concentration and lactate/pyruvate ratio. However, since there were no differences in the adenylate energy charge, the fall in phosphocreatine/creatine ratio may have been due to acidosis (Siesjö *et al.* 1972). The ammonia concentration was increased in the hypoxic and hyperthermic groups.

The present results demonstrate that when sustained epileptic seizures are complicated by moderate degrees of arterial hypotension or hypoxia, or by *hyperthermia, cellular lactic* acidosis is exaggerated, and there is further accumulation of ammonia. However the phosphorylation state of the adenine nucleotide pool is maintained at values obtained in uncomplicated status epilepticus. *It remains to be shown if the complicating factors, studied* presently can aggravate the histopathologic lesions occurring. *If this is so,* the mechanisms should be unrelated to a general deterioration of cerebral energy state.

The authors are grateful to Kerstin Belrup for skilful technical assistance. This study was su[illegible] grants from the Swedish Medical Research Council (project No. 14X 263-13), from U.S. PHS [illegible] 5 R01 NS07838-08 from N.I.H., from Föreningen Margarethahemmet and Förenade Liv

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Acta physiol. scand. 1977. 100. 129–138
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Tail Artery Response to Sound in the Unanesthetized Rat

by

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Received 28 June 1976

Abstract

Borg, E. *Tail artery response to sound in the unanesthetized rat.* Acta physiol. scand. 1977. *100.* 129–138.

Arterial pulsations have been recorded indirectly from the surface of the rat' tail. Slight heating of restrained unanesthetized rats produces vasodilatation and large amplitude pulsations that are influenced by sensory stimuli, in this case, sound. The surface-recorded pulse volume was found to be proportional to pulse pressure, indicating vasoconstriction as the cause of the decline of the pulse amplitude. A one-second noise burst elicited vasoconstriction, the duration of which was proportional to sound level and occurred at low as at hearing threshold. Under the specified conditions, reproducibility was good with no significant habituation both within sessions, and between sessions with one week interval. The heating of the rats was found to be critical for the sound-elicited reactions: responses were obtained only within a narrow individual temperature-range. The possibilities of using tail vasoconstriction for evaluation of hearing is pointed out, as well as for studies of noise effects on peripheral circulation.

The capability of acoustic signals to influence cardiovascular homeostasis has long been known. A decline of hand volume during sound exposure was observed as early as 1874 (Mosso): a possible differential effect on heart rate and blood pressure of a Tschaikowsky symphony of a death-like character and of the musical rendition of a bullfight in Carmen was noted in 1918 (Hyde and Scalapino). During the last decades, cardiovascular effects of sound have again been observed and analyzed in more detail. A significant rise in blood pressure upon exposure to bursts of loud noise was found to occur in unanesthetized, spontaneously hypertensive rats, but not in normotensive ones (Hallbäck and Folkow 1974). Cutaneous vessels in various regions constrict or dilate even at low sound levels (e.g. Sokolov 1963, Jansen 1974), and heart rate may either increase or decrease (Sokolov 1963, Inglis 1974, Berg *et al.* 1975). Though it is now well established that sound may elicit cardiovascular reactions, scant attention has been given to the quantitative relation between the response, e.g. its amplitude or duration, and values of sound parameters (exception being Jansen *et al.* 1964, Jansen 1974). If a stable relation of that kind could be determined, it might be useful in evaluating central auditory mechanisms as well as physiological effects of various types of environmental noise.

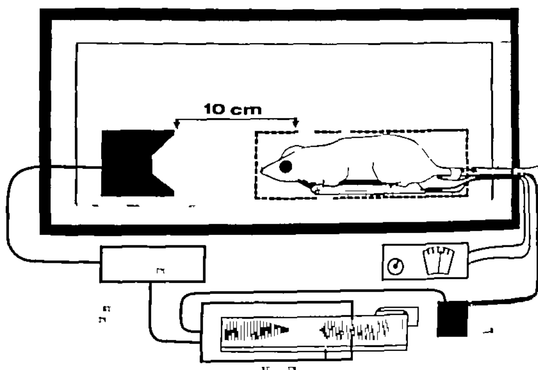


Fig. 1 Schematic view of experimental situation.

The purpose of the present paper is to describe how arterial pulsations in the rat s tail are influenced by acoustic stimuli in the intensity range from hearing threshold to high level. A technique will be presented for recording arterial pulsations in the rat s tail and the conditions established under which stable vascular reactions are obtained in response to sound stimulation. The vasoconstriction is quantified in terms of *duration* of the decline of pulse amplitude No previous description of the influence of sound on circulation in the rat s tail has been presented.

Methods

The vascular reaction of the tail to sound was examined in 43 experimentally naïve Sprague-Dawley rats of both sexes. The majority of these were 3–5 months of age seven rats were aged from 15–18 months. Twelve additional ad lt rats were used fo control measurements

Arterial pulsations V lume pulsations were recorded from the surface of the tail of the slightly restrained unanesthetized rat. A rise of mbient temperature was necessary since the tail rtery is constricted at room temperature and dilates only i the process of temperature regulation. The experimental situation is schematically shown i F g 1 The rat rests in an individually adjustable net t be. A thermistor probe and a small heating pad were introduced below the rat the tube. Temperature was regulated w th 0.1 (Antern) and was usually with n the 37 38°C ra ge for ptimal tail vasodilatation The surrounding sound-insulated chamber was ventilated with 30°C ir A small rubber balloo w t ped t the base of the ventral side of the ta l. It was connected t a volume-sensitiv transducer (Elema 510 C) by a 0 4 m long flexible t be. The tube was penetrated by 1 -gauge cannula in order t decrease low frequency disturbances due to tail movements. The pulsations, together with temperature nd timulus indicator signal, were recorded on an Elema 34 T ink-recorder

A oustic stimulation. Stimulus noise was generated by General Radio 1381 random noise generator (2 H 50 kH) and presented through Quad 303 power mplifier t Lancing L75 horn placed 10 cm i front of the rat ea plane (F g. 1). The signals were presented i burst ration wit

decay times (Forsima delay and gate units). For the broadband signal used, the leakage in the gate as 65 dB. Sound levels were measured in the rat tube at the rat ear plane with 1/4 microphone (No 4135) and 2606 measuring amplifier through 1/3 octave band filter all instruments made by Brüel & Kjaer Fig. 2 shows the sound pressure level (SPL) of stimulus noise as function of the center frequency of the 3 octave bands (in order to obtain noise spectrum, subtract 3 dB octa). The maximal 1/3 octave band level as around 6 kHz.

Procedure. The rats were trained to rest in the rat tube for 1 h day during 5 days. A rule the animals rapidly accepted the restion and ere often found sleeping at the end of the training session. I the experimental session the animal as placed in the tube on the regulated heating pad and gently warmed until exhibited clear vasodilatation, indicated by more than 10-fold increase of pulsation amplitude from the unheated level. The temperature had to be increased up to this point slowly since too rapid heating may be painful and make the rat uncomfortable. It may also result in too high degree of body heating, and consequently vasodilatation so pronounced that no sensory stimulation, not even painful one, could affect As soon as stable vasodilatation as obtained, "reference signal" noise 80 dB SPL, was presented. Usually clearcut vasoconstriction of several tens of seconds duration as obtained (see Fig. 3). If no such response as obtained, the temperature as lowered few tenths of degree and the stimulus repeated after about 15 min. This procedure has up to now never failed with experimentally naive rats. When the response to the reference sound was obtained, noise bursts different levels were examined at random order. Intervals between the presentations were kept at 10–15 min. If, however the recording showed artifacts due to movements, the stimulus as postponed until the recording as distortion-free. In order to obtain running check of experimental conditions, every second stimulus was the reference noise (80 dB SPL). This control procedure was instituted only on the last 14 animals of the series. The stimulus noise signals were kept between 5 and 90 dB SPL. Since the leakage of the gate was not better than −65 dB, the highest levels were accompanied by an audible sound in the interstimulus interval. The rat had approximately 15 min to adapt to this sound.

In short, the following precautions ere observed in order to get high degree of stability of the responses.

1) The interstimulus interval should be at least 10 min.
2) The sound should be presented only hen the rat exhibits regular pulsations free from movement artifacts
3) The rat should not be overheated.
4) The latent time for vasoconstriction should be no longer than 2 sec.
5) Recordings here the sound-elicited vasoconstriction supercedes by subsequent spontaneous reaction should be discarded

Control experiments. Blood pressure and pulse pressure were recorded from the central tail artery by an intra-arterial catheter (PE 50 or PE 90) connected to Statham P 23 AC transducer (3 animals). Indirect volume pulsations ere recorded as described above from sensor placed central to the catheter. The rats were gradually armed to produce arious degrees of vasodilatation. The pulsation amplitudes obtained by the two methods ere compared. The operations were performed ith the animals in ether or nembutal anesthesia. The recordings were obtained during anesthesia and after hen the animal as behaviorally awake.

Flow rate in the tail artery was measured in three animals ith Parks 803 ultrasonic flow meter. The amplitude and temporal course of the pulsations in flow rate ere compared to the ohmic pulsation simultaneously recorded from sensor distal to the ultrasonic probe.

The influence of heating pad temperature on tail vascular response to sound as investigated in 6 rats. The temperature of the pad was raised or lowered in small steps, whereafter the rat equilibrated for 15 min. At the end of this period was exposed to 1 80 dB SPL noise burst. The response duration at various pad temperatures as observed in order to establish the optimal degree of heating.

Results

A. *Basic characteristics of sound-elicited vascular reaction*

When a rat was adequately heated, the tail artery dilated and a sensory stimulus was usually followed by a decrease in pulse amplitude. Fig. 3 shows recordings of pulsations from the

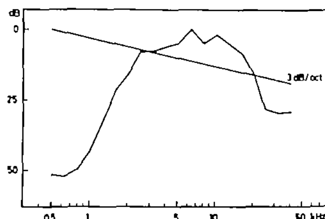


Fig. 2. Characteristics of stimulus are as measured with a 1/3-octave filter. Spectrum level is obtained by correction according to the broken line (3 dB octave).

surface of the tail and the influence thereon of a 1 s burst of broadband noise at 3 sound levels. The recordings were obtained in the same experimental session in one rat. Pulse amplitude decreased after a latency of about 1 s. The minimal amplitude was often only 1 % of the prestimulus amplitude and the duration of the response lasted up to about 1 min.

There are many possible ways to quantify a vascular reaction of the type shown in Fig. 3. The most attractive way might be to measure the amplitudes A and B (of Fig. 3) and to calculate e.g. B/A 100 per cent or $(1-B/A)\times 100$ per cent. Fig. 4 shows the average of $(1-B/A)$ 100 per cent as a function of sound level of a one second broadband noise in 1[?] rats. The experimental values follow a clearly nonlinear intensity function with a saturation above 50–60 dB SPL sound. Such a nonlinearity is, however expected on the basis of the Poiseuilles law which states that the flow is related to the radius of the vessel raised to the fourth power As long as the decline of radius during sound stimulation is small the intensity function is expected to approximate a straight line but when the vascular reaction becomes prominent the curve gradually would deviate from linearity The dotted line of Fig. 4 shows the approximation of a function containing a term raised to the fourth power ($y = a + b(c-x)^4$) to the experimental values. By a nonlinear regression technique the constants a 87.0 b = −0.16 10^{-4} c = 150 have been found to give an acceptable description of the

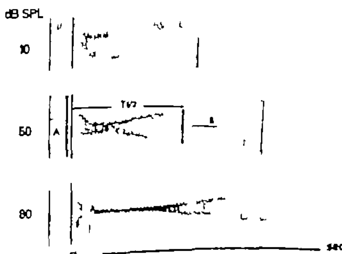


Fig. 3. Pulsations recorded from the surface of the tail of an unanesthetized rat. A one-second noise burst elicit a decline of pulsation amplitude, the degree and duration of which depend on sound level. The vasoconstriction is quantified by $T_{1/2}$, the time from stimulus onset to recovery to $(A+B)/2$, where A is pre-exposure amplitude and B is minimum pulse amplitude.

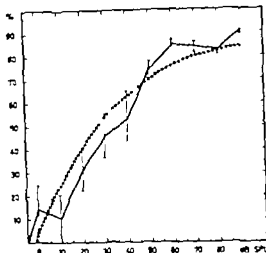


Fig. 4. Decline of pulse amplitude after 5 sec tone bursts of various sound levels. Ordinate [illegible] (1 − B/A) · 100 per cent, where A is the [illegible] pulse amplitude and B is the [illegible] poststimulus amplitude. The dotted line shows the adapted function y = [illegible] + b [illegible] 57.0 [illegible] 0.16 · 10⁻⁴ [illegible] −150. Average of measurements in 14 rats.

Experimental results. "y" is the percentage decline of pulse amplitude during sound exposure, "a" corresponds to the initial pulse amplitude, "c" corresponds to the initial radius of the vessel and "x" the decline in radius caused by the sound stimulation, "b" is a constant. As is seen in Fig. 4 the fit is quite good, indicating that the decline in pulse amplitude is caused by an appreciable decline in vessel radius during sound stimulation.

If the purpose had been only to analyze the nature of the response, the amplitude would probably be the best parameter to measure. If, however, as in the present study, the main purpose is to obtain a measure that can be used to compare various sounds with respect to their influence on a peripheral vascular system, a parameter with a more linear relationship to sound level would be to prefer.

The duration of the response was found to be such a parameter. The time which elapsed from the onset of the sound to the moment when the pulse amplitude had halfway returned to its initial value ($T_{1/2}$ of Fig. 3) was used as a measure of the vascular reaction (vasoconstriction, see below).

At low sound levels no movement-induced artifact was superimposed on the recordings at the beginning of vasoconstriction. At the high level (90 dB SPL) such influence was often, but by no means always, seen. On the other hand, movements without concomitant vasoconstriction were often evident. It is to be noted that even minute movements were observed as disturbances in the recordings.

The recordings in Fig. 3 are selected to approximate the average response characteristics at various sound levels. There was, however, a considerable variation in the response magnitude, inter- and intraindividually in spite of the precautions described under Methods. Fig. 5 shows individual stimulus–response curves obtained in 14 of the 23 experimentally naive rats and the corresponding average curve. Only those rats were included which were subjected to the running control with the 80 dB reference sound. Single curves vary greatly in shape. The average stimulus–response curve was, however, approximately a straight line up to 30 dB. Its threshold was somewhat below the behaviorally determined threshold for rats (Barrow, Gourevitch 1965). Detailed comparisons on this point are, however, impossible.

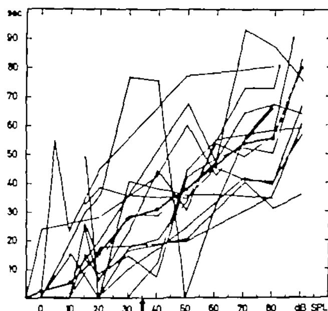


Fig. 5 Duration of vasoconstriction as function of the level of a 1 s noise burst (stimulus-response curves) in 14 rats. At 80 dB, up to 10 responses were obtained for each animal; at other levels [illegible] determinations were available. Solid line shows average calculated for each 10 dB step. Arrow shows hearing threshold obtained by traditional behavioral technique (Gourevitch 1965).

B *Validity of indirect pulse recording*

The surface-recorded pulsations (indirect) have been viewed in relation to the pulse pressure directly obtained intra-arterially distal to the site of the volume pulse recording. In all of the 9 simultaneous recording series of this type, the relation was closely linear with correlation coefficients between 0.71 and 1.0 (median 0.96). The peak amplitude of the volume change due to the arterial pulse wave was between 0.0[illegible] and [illegible] mm³.

Simultaneous recordings of pulse volume and pulse pressure were also made during noise exposure. A noise burst elicited a decline in pulse pressure amplitude as well as in pulse volume of approximately identical time courses. This similarity also indicated that the relation between pressure and volume in these recordings is linear.

The relation between flow rate and pulse volume amplitude was less clearcut than that between pulse pressure and pulse volume. The flow rate peak occurred, as expected, before the peak of the pulse volume. The relation between peak amplitudes, however, was, as a rule, nonlinear.

After sound stimulation, the pulsations of flow rate were sometimes seen to decrease and sometimes to increase, whereas pulse volume regularly decreased. The relation between pulse volume, pulse pressure and flow rate, points to vasoconstriction rather than shunting as an explanation of the decline of pulse volume amplitude (see also Discussion).

C. *Reproducibility*

In the stimulus paradigm applied in this study every second noise burst was at the reference level (80 dB SPL). These signals were usually presented at intervals greater than 1/2 h throughout each experimental session. The reproducibility within a session was evaluated on the basis of the responses to these reference stimuli. Fig. 6 shows individual regression lines calculated on the basis of 13 expts., including 4–10 stimuli at reference level. The slopes varied between −0.017 and +0.016 with an average of −0.004. With regard to the generally small slope-constants and the horizontal course of the average curve, one may conclude that habituation is not a prominent feature of vasoconstriction (to 80 dB SPL noise) under the prevailing circumstances.

The short term reproducibility (Fig. 6) is, however, dependent on how well certain interfering factors are controlled. Ongoing or phasic motor activity may bring [illegible] spontaneous vasoconstrictions or a decrease

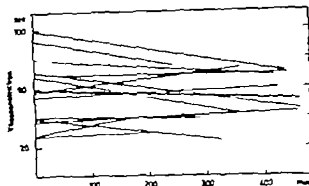


Fig. 6. Short term stability of vasoconstriction elicited by 80 dB SPL noise bursts in 13 rats. Regression lines are based on 4–10 exposures within a time range of up to 8 h from first to last stimulus. Heavy line shows average based on individual slope and intercept.

of sound-elicited response. Spontaneous vasoconstriction occurred both in the absence and in the presence of observable movement artifacts. Such spontaneous reactions occasionally interfered with sound-elicited responses. Responses of such bimodal or multimodal character were excluded.

The ambient temperature resulting from the heating necessary to produce a basic degree of vasodilatation may be expected to bear upon the size of the sound-elicited vasoconstriction. Fig. 7 shows duration of vasoconstriction in response to 80 dB noise bursts in one typical experiment at various pad temperatures. At low temperature the artery was not dilatated and thus no vasoconstriction could be recorded in response to sound. Within a narrow temperature range, stable vasoconstriction responses to the 80 dB stimuli were obtained. When the rat was heated to about 1 degree above optimum, it no longer responded with vasoconstriction to an 80 dB noise burst.

Heart rate was seen to increase during motor activity and at high temperature. A high heart rate thus may generally be expected to be accompanied by small vasoconstriction. Even when precautions were taken as to temperature and motor activity as in the present experiments, certain variation with respect to both response duration and heart rate remained. In some cases, decline of response duration was seen as a function of heart rate. Among the animals with rank order correlation coefficient higher than 0.4, the majority (10 of 11) showed negatively sloping regression line. The average slope was only −0.09 implying shortening of duration by 0.9 per 10 beats/minute increase in heart rate. This weak correlation does not motivate consideration of heart rate other than that implicit in the control of temperature and movement artifacts.

Granted the precautions taken short term reproducibility hopefully is acceptable to reveal the role of sound properties for the degree of tail vasoconstriction.

Reproducibility over a longer time period, one week, was investigated in seven rats. A stimulus–response relation for 1 noise bursts at various levels was determined in two separate sessions, one week apart. In the latter session, it usually took longer (though less time, in one case) to acclimate the rat to the measuring situation. After a delay of up to several hours, five of seven rats relaxed and the measurements could be continued in the usual manner. The two remaining rats were excluded when they failed to relax sufficiently to meet the requirements of distortion-free pulse recording after approximately 3 h.

Reproducibility obtained under these conditions is illustrated in Fig. 8. The solid line represents the average initial stimulus–response curve (5 rats), and the corresponding broken-

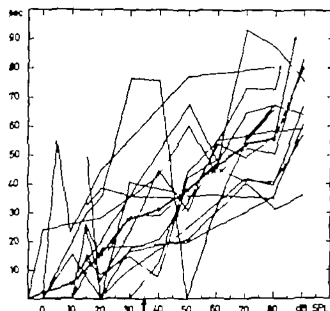


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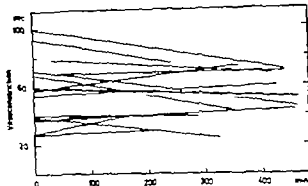


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Heart rate was seen to increase during motor activity and at high temperature. A high heart rate thus may generally be expected to be accompanied by a small vasoconstriction. Even when precautions were taken as to temperature and motor activity as in the present experiments, a certain variation with respect to both response duration and heart rate remained. In some cases, a decline of response duration was seen as a function of heart rate. Among the animals with a rank order correlation coefficient higher than 0.4, the majority (10 of 11) showed a negatively sloping regression line. The average slope was only −0.09, implying a shortening of duration by 0.9 s per 10 beats/minute increase in heart rate. This weak correlation does not motivate a consideration of heart rate other than that implicit in the control of temperature and movement artifacts.

Granted the precautions taken short term reproducibility hopefully is acceptable to reveal the role of sound properties for the degree of tail vasoconstriction.

Reproducibility over a longer time period, one week, was investigated in seven rats. A stimulus–response relation for 1 s noise bursts at various levels was determined in two separate sessions, one week apart. In the latter session, it usually took longer (though less time, in one case) to acclimate the rat to the measuring situation. After a delay of up to several hours, five of seven rats relaxed and the measurements could be continued in the usual manner. The two remaining rats were excluded when they failed to relax sufficiently to meet the requirements of distortion–free pulse recording after approximately 3 h.

Reproducibility obtained under these conditions is illustrated in Fig. 8. The solid line represents the average initial stimulus–response curve (5 rats), and the corresponding broken-

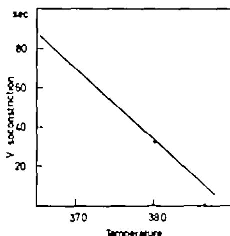


Fig. 7

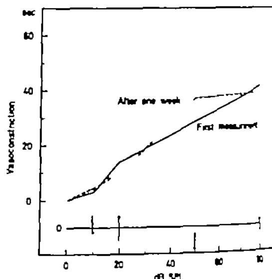


Fig. 8

Fig. 7 Duration of vasoconstriction as a function of heating-pad temperature. One experiment, 80 dB SPL noise bursts. Regression line is shown by continuous line.

Fig. 8 Reproducibility of vasoconstrictions over one week period elicited by 1 s noise bursts at various levels in five young rats. Insert shows average (O) and standard error of the mean of individual difference between first and second measurement.

line the curve (5 rats) obtained after one week. A close correspondence between these average curves is evident The averaged difference between corresponding measurements in each individual rat is shown in the insert at the bottom of the graph Each point shows average individual difference and standard error of the mean.

Discussion

The observations presented show that sound stimuli in a wide range are capable of eliciting a decline in pulsation amplitude recorded from the surface of the rat s tail. The decline of pulse amplitude followed a nonlinear intensity function, saturating for the noise above 60 dB SPL. For the purpose of comparing the influence of different sounds, amplitude is thus not well suited The duration of decline of amplitude was, however on the average linearly related to sound level above hearing threshold. This fairly stable relation to sound intensity became a reality when experimental conditions were standardized with respect to stimulus interval temperature and motor activity of the animals

Simultaneous recordings of pulse volume and pulse pressure showed a linear relation Consequently pulsations decreased in both volume and pressure upon sound stimulation, implying that the decrease in pulse volume was due to vasoconstriction and not to shunting at the base of the tail. Pulsations in flow rate were even seen to increase concomitantly with a decrease of pulse volume In those instances, there might have been a local vasoconstriction near the site of the recording, controlling flow in the more distal parts of the tail

Graded vasoconstriction in response to sound has not previously been described in unanesthetized animals. Peripheral vascular reactions to emotional ousal stimuli in-

uding noise have, however been analyzed in some detail in animals (Abrahams *et al.* 1964 olme *et al.* 1967 Caraffa-Braga *et al.* 1973). In man, pulsations in digital blood supply ave been recorded by a plethysmographic technique for the purpose of evaluating influence f sound on vegetative functions (e.g. Jansen *et al.* 1964 Jansen 1974). The decline of finger ulse amplitude is, however small and occurs only with relatively high level sound (70 dB PL). Recent work indicates that vasoconstriction in fingers (Jansen *et al.* 1964, Jansen 1974) s ell as in the rat s tail depends on sound parameters and increases as a function of sound vel.

The reflex nature of the sound-elicited vasoconstriction is obscure. Even though it may e relayed basically over a simple brain stem reflex mechanism, it is most likely controlled y higher nervous centers, e.g. the hypothalamus (Abrahams *et al.* 1964, Bolme *et al.* 1967) nd the orbital cortex (Petřek *et al.* 1970) The asoconstriction is, however more related o the sound-elicited changes in galvanic skin resistance (e.g. Barr 1955, Berlin 1963), heart ate, pupil diameter and respiratory rate than to the acoustic stapedius muscle reflex. These ormer reactions are variable and are generally known to habituate rapidly whereas the atter is stable and exhibits a graded response amplitude in relation to sound level, although t has a high threshold (see e.g. Borg 1972) Due to its stability the acoustic stapedius muscle eflex has gained considerable use in clinical diagnosis of auditory disorders (e.g. Klockhoff 961, Anderson 1969 Lidén 1970) and in experimental auditory research (Borg 1973). The rocedure for recording arterial pulsations in the rat s tail keeps habituation of vasocon-striction and its variation within reasonable limits. Hopefully thus reflex mechanism will rove useful for auditory measurements in the conscious rat. The graded nature of the vaso-constriction and its high sensitivity to sound stimulation offer advantages over conditioned eflexes and stapedius muscle reflex.

Since sound is evidently capable of eliciting strong vasoconstriction in at least some periph-eral vascular beds (tail, finger), the question of the significance of these effects over long me periods arises. Can vascular homeostasis be altered and local blood flow or systemic blood pressure influenced temporarily or permanently? In recent studies in the unanesthetized at, evidence for general effects of short-term sound exposure on blood pressure has been obtained (Hallbäck and Folkow 1974). The significance of environmental sound during very long periods for cardiovascular functions is still largely unknown.

In conclusion, sound was capable to elicit vasoconstriction in the rat s tail, the duration of which depended on the intensity of the sound. Variation in responses obtained was kept acceptably low by controlling temperature, stimulus interval and regarding the rat s motor activity Experiments are now in progress to evaluate the role of other sound characters on the degree of vascular response.

This study as supported by the Swedish Work Environment Fund Project nr 74/24

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Acta physiol. scand. 1977. 100. 139–153
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The Compliance Curve for the Flow Limiting Segments of the Airway

II. Experiments with human subjects

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Received 2 July 1976

Abstract

PEDERSEN, O. F. and T. M. NIELSEN. *The compliance curve for the flow limiting segments of the airway. II. Experiments with human subjects.* Acta physiol. scand. 1977. *100.* 139–153

Maximum effort flow-static recoil curves were obtained in 5 healthy subjects breathing air, He/O_2, and SF_6/O_2 mixtures. In 4 of them maximum effort flows corresponded to really maximal flow, and their curves were transformed into compliance curves for the flow limiting segments of the airway and analyzed from the point of view of a previously presented lung model (Pedersen and Nielsen 1976). The results showed that density dependent pressure losses from the alveoli to the flow limiting segments were minimal for air and SF_6/O_2, but not for He/O_2. When viscosity dependent pressure losses could be neglected, then expiration of gases of different densities gave almost identical compliance curves for the flow limiting segments. This supported the applicability of the model. The calculated compliance curves for the flow limiting segments were compared with data from the literature, and the findings indicated that flow limitation during expirations with just maximal flows throughout began in the extrapulmonary airways and moved upstream during the expiration.

Key words: Mechanics of the expiration, airway compliance, inert gases, maximum flow—static recoil curves

The purpose of the present investigation has been to evaluate in experiments with human subjects some theories, tested previously on a mechanical model of the lungs (Pedersen and Nielsen 1977).

In accordance with these theories the compliance curve relating *transmural pressure* (Ptm) and cross-sectional area (A) of the flow limiting segments of the airway can be calculated from the relationship between the elastic recoil pressure of the lungs (Pel) and the maximal expiratory flow (Vmax). Provided static recoil pressures (Pst) and dynamic recoil pressures can be considered equivalent, this relationship can be obtained in humans (the maximum flow—static recoil (MFSR) curve of Mead *et al.* 1967).

The relationship between Ptm and A, determined from the MFSR curve reflects the elastic properties of the airway where and when flow is limited. To be more exact, it is the compliance curve for a single elastic airway behaving in exactly the same way as the complex

system of branched elastic tubes forming the human intrathoracic airways, and does *per se* say anything about the site of flow limitation.

The equations used in the transformation of the MFSR curve into the compliance cur for the flow limiting segments of the airway are

$$A = \sqrt{2c \;\; \dot{V}max \;\; \frac{d\dot{V}max}{dPst}} \qquad (1$$

and

$$Ptm = Pst - \frac{c}{A} \, \dot{V}max^{2} \qquad (2$$

where c is a constant depending on the density of the expired gas $= 10^{6}\,\varrho/^{?}G(Cc)^{2}$ (g cm s), ϱ being the density of the gas (g cm^{-3}) G the acceleration of gravity (981 cm s^{-2}), and Cc a constant depending on the flow conditions in the airway The latter was in all the experiments assumed to be equal to one as found for the converging part of the airway of the mechanical model (Pedersen and Nielsen 1976). Vmax is measured in l s^{-1} Pst and Ptm in cm H O and A in cm dVmax /dPst is the slope of the MFSR curve at the pre Vmax The mark ( ) indicates that the parameter is valid where and when flow is maximal in the airway In some cases there is a decrease in flow at a given Pel when the transpul monary pressure (Pl) exceeds a certain optimal value (Pl) (Fry and Hyatt 1960, Mead et al 1967) Maximum effort flow (Vmax) then becomes less than maximal flow ($\dot{V}$max), a phenomenon called negative effort dependence"

If the Ptm –A relationship is solely determined by the elastic properties of the airway it should not be affected by the properties of the expired gas.

The purpose of the first part of the experiments has therefore been to compare Ptm curves calculated from MFSR curves obtained during expirations of gases with differ physical properties to see whether viscosity dependent pressure drops, which were not in corporated in the equations, would invalidate their use, and whether change in density of the expired gas would change the Ptm –A relationship and thus invalidate the basic con cepts of the applied model

The purpose of the second part of the experiments has been to examine whether the max mum effort flow (Vmax) used in the expts. above equalled maximal flow (Vmax). Becau only if negative effort dependence could be ruled out the equations could be applied t maximum effort flows.

Preliminary expts. with the mechanical lung model (Pedersen and Nielsen 1976) ha shown that Vmax at a number of different recoil pressures could be obtained by the metho of Olafsson and Hyatt (1969) from expirations through stenoses of different sizes, and th method has also been used here.

If the equations could not be proven to be invalid, *the final purpose of the present study* h been to evaluate the results in the light of the equal pressure point concept of Mead et (1967), and furthermore to compare the Ptm –A curves with curves calculated for differe airway generations *in vitro* in search for the approximate location of the flow limiting se ments in the airway

Methods and Material

...e young healthy subjects (1 female and 4 males) were examined.

Using an N_2-dilution method (closed system) the total lung capacity (TLC), the residual volume (RV), and the "closing volume" (CV) were determined and expressed at BTPS.

Then the mucosa of the nose and the pharynx was anaesthetized by means of spray containing 5 mg lidocaine. An oesophageal balloon, length 10 cm, perimeter 5 cm, mounted on 1 mm bore plastic tubing was then introduced through one of the nostrils and positioned in the lower third of the oesophagus. The balloon was subsequently evacuated to pressure of 10 cm H_2O, and 0.5 ml of air was introduced.

The subject was then seated comfortably in front of wide bore (28 mm internal diameter) mouth piece, which could be obstructed to various degrees by insertion of slide with holes of various sizes. From this mouthpiece the subject breathed through heated pneumotachograph (Fleisch no. 4). Between the pneumotachograph and the mouthpiece an electric shutter was inserted. It could be operated automatically. The pneumotachograph is connected to a differential transducer (EMT 32C) and the signal amplified in an electromanometer (EMT 311). The flow signal from here was integrated in an integrator constructed in the workshop at the institute, and the integrated flow signal (i.e. the volume signal) was recorded together with the flow signal on 6-channel jet ink recorder (Mingograf 81, Elema Schönander, Stockholm).

The pneumotachograph was calibrated in the interval from zero to 15 l s^{-1} for the use of atmospheric air, mixture of 20 oxygen and 80 helium, and mixture of 25 oxygen and 75 sulfahexafluoride (SF_6).

The pressure difference (Pl) between the esophageal balloon and the mouthpiece at the oral side of the ... holes was measured, and so was the pressure difference between the esophageal balloon and the atmosphere (Ppl).

The pressure-measuring equipment consisted of two transducers (EMT 35, Elema Schönander) with electromanometers (EMT 311). One transducer was connected to the esophageal balloon and the other to the mouthpiece, both having the atmosphere as the reference. Pl was obtained by electronic subtraction of the outputs from the two manometers and was recorded together with Ppl, and the flow and volume signals from the pneumotachograph.

Static P–V curves of the lungs

Connected to the mouthpiece with no resistor inserted and the shutter open, the subject was asked to breathe normally. After about 5 normal breaths the subject was asked to breathe in maximally and hold the breath for a short while. The subject then exhaled slowly to RV for 10 to 15 s, while the shutter automatically interrupted the gas flow for 0.6 s after each second of flow. With the shutter turned off the subject was then asked to breathe normally again. The procedure was repeated until 5 sets of curves without artefacts were obtained. At each interruption of the gas flow the volume V in the lungs above RV and the elastic value of Pst ... Pl ... are measured. Static pressure-volume curves for the lungs were drawn as the best fitting curves through points plotted from these values.

Concluding the main experiments a new series of static P–V curves was performed. Average values of the initial and final results were used in the computation of the MFSR curves and derived results.

Maximum effort flow curves

The subject then performed a series of forced vital capacity manoeuvres directly through the pneumotachograph, and flow ($\dot{V}$), volume (V) and pleural pressure relative to mouthpressure (Pl) and to the atmosphere (Ppl) were recorded continuously. The corresponding Pst at any volume could be obtained from the static pressure-volume curves. Due to compression of the gas in the lungs, however, this elastic pressure is overestimated (Ingram and Schilder 1966). Therefore corrected Pst was obtained from the Pst–V curve by substituting V for the reference volume V meaning

$$V = \frac{B \cdot V - RV \cdot Palv}{B - Palv} \quad (3)$$

(cf. Appendix)

In this equation, B is the barometric pressure (cm H_2O), RV is the residual volume of the lungs determined by way of introduction. Palv is the sum of the pressure drops from the alveoli to the mouth and from the mouth to the atmosphere. In equation 3, Palv = Ppl – uncorrected Pst, because the actual Pst, which should be used, is the parameter we are looking for. Using the uncorrected Pst instead, ... causes

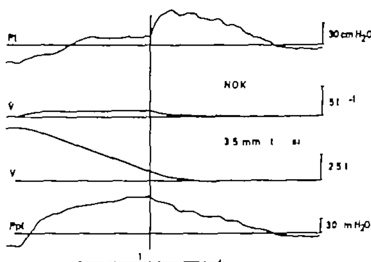


Fig. 1 Recordings of Pl, V̇, V, Ppl during a forced expir from TLC to RV by subject N The expiration is perfor through a 3.5 mm stenosis m mouthpiece. V̇max is reache the vertical line. From here decreases in spite of an ab increase in Pl (cf text)

a minimal erro when Ppl is large and contributes the major part of Palv When Ppl is small, the will also be small due to minimal compression of the gas.

Corrected values of Pst were calculated at regular V̇max intervals beginning at the peak expiratory and yieldi g 5 to 7 points in each expiration. From such points obtained in 4 to 5 expirations, a curve was drawn as the best f tting curve by eye. At 15 to 20 points along this curve, the slope dV̇/dPst was measured together with V̇max and Pst. Assuming a value of c = 0.580 g cm^{-4} s^{2} for air the c sectional area (A) and the transmural pressure (Ptm) were then calculated for each of these points b of equations 1 and 2, in spit of the fact that these equations are only valid when V̇max = V̇max' (cf ab The best fitti g curve by eye was then drawn through the calculated points.

After the f rced vital capacity manoeuvres in air the subject was connected to a bag containing a m of 20 % oxygen in helium. The air in the lungs was washed out by 5 deep breaths of this mixture, series of forced vital capacity manoeuvres with the new gas was done. MFSR curves were plotted as d In calculating the relationship between Ptm and A, we here used value of the constant c ρ 10 0 19 g cm^{-4} where ρ was the volume weighted density verage fo o ygen and helium at 37°C.

Finally the procedure was repeated with the SF_6/O_2 gas mixture. The value of c for this gas m was 2.38 g cm^{-4} s^{2}

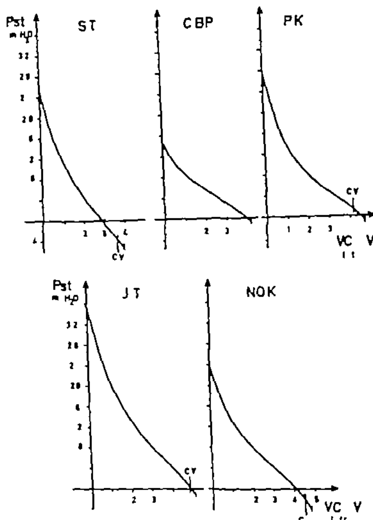
Optimal curves

For determination of the just maximal flow (V̇max') and the corresponding elastic pressure (Pst') orifice method of Olafsson and Hyatt (1969) was used. V̇max' should occu at the moment when the pr difference between the nside of an esophageal balloon and the mouth (Pl) becomes equal to a c optimal value (Pl') and suddenly begins to increase. The method is illustrated by Fig. 1 At the line Pl becomes larger than a certain value Pl and starts to increase rather suddently due to compr of the airways. The corresponding flow is the just maximal flow V̇max The orifices used in the p expts. had diameters between 3 5 and 23 mm. The subject performed 2 forced vital capacity manoe through each of 5 different stenoses giving 10 sets of related values of Pst and V̇max'. Also in these Pst was corrected fo alveolar compression. These experiments were only performed after breathin

V̇max was plotted as a function of Pst' and the relationship between Ptm' nd A calculated as

TABLE I Vital statistics and lung volumes of the subjects in the study Lung volumes are corrected to

Subject	Sex	Age	Height cm	Weight kg	TLC l	RV l	VC l	C l
ST	M	18	165	56	4 91	0.96	3.95	0.2
CBP	M	21	172	55	5.57	1.40	4 16	–
PK	F	20	171	62	5.51	0.91	4 60	0 4
JT	M	22	185	81	5 91	0.76	5.15	0.2
NOK	M	22	171	62	6 09	1.27	4.82	0 3



ig. 2. Relationships between expired volumes from TLC and Pst in the 5 subjects. The curves terminate at RV. CV indicates the "closing point" (cf. text).

Results

olumes and capacities

n Table I, the pertinent data concerning the subjects investigated are presented. The values of TLC, RV, VC, and CV were calculated as averages from 2 expts.

The relationship between V and Pst

n Fig 2, Pst has been pictured as a function of the expired volume from TLC (= VC–V) or the individual subjects. The closing volumes (CV) are marked on the curves. The alues of Pst become negative in all cases. This finding will be discussed later

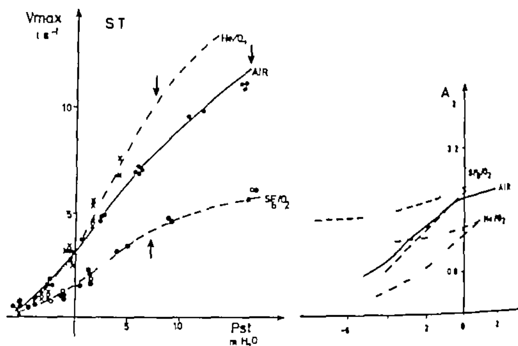


Fig. 3

Fig. 3–6. *Left or upper parts.* The relationships between Pst and maximum effort flow (Vmax) for different gas mixtures and subjects (except CBP). Points from 5 expirations of each gas mixture are plotted and the curves best fitting by eye are drawn through the points. The arrows indicate the points where ... is most positive. *Right or lower parts.* The derived relationships between Ptm and A (*cf* text)

The relationships between Pst and Vmax (from maximum effort flow and volume curves) and the derived Ptm–A relationships

Fig. 3–6 illustrate the relationship between Pst and Vmax for the individual subjects (except CBP *cf* below) and the different gas mixtures. Underneath or next to these curves are plotted the derived relationships between Ptm and A calculated on the assumption that V̇a = Vmax (*cf* above) from equations 1 and 2.

It is seen that at a given Pst Vmax for SF_6/O is on the average about 50 % of Vmax for air. At high values of Pst, Vmax for He/O is considerably greater than Vmax for air, at lower Pst values the two curves intersect in 4 of the 5 subjects.

Most of the Ptm–A curves consist of 2 portions forming an angle open to the left. The course during the expiration is thus very similar to what was seen for the mechanical ... (Pedersen and Nielsen 1973). It begins to the left and ascends along the upper side of the angle towards its top point (corresponding to the arrow at the Pst–Vmax curve above) and then continues downwards to the left along the lower side of the angle.

Immediate inspection of the Ptm–A curves for air and SF_6/O shows that there is ... systematical difference. The curves for He/O give as a common trend lower values ... for given values of Ptm.

The relationship between Pst and Vmax (from optimal curves) and the derived Ptm ... relationship

Maximal and maximum effort values of V were equal in all subjects except CBP. Here there was a disagreement, which is shown in Fig. 7 where the ... in the left

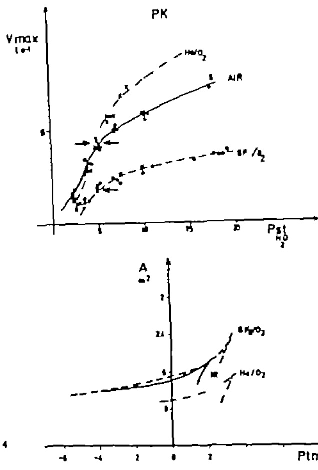


Fig. 4

...of the figure illustrates the maximum effort Pst $\dot{V}$max relationship and the broken curve the ...Pst $\dot{V}$max relationship On the right diagram are drawn the corresponding Ptm–A ...and Ptm–A curves. Because of the disagreement, the maximum effort curves of CBP were considered unsuitable for the purpose and discarded.

Discussion

The purpose of the first part of the expts. has been to examine whether viscosity dependent pressure drops would invalidate the use of the equations, and whether change of density of the expired gas would change the Ptm A curves.

In the expts. with the different gas mixtures we measured maximal effort flow ($\dot{V}$max) and not just maximal flow ($\dot{V}$max'), which should be used in the equations. Therefore it was necessary to check that these two flows were indeed equal. This was the case in 4 of the 5 subjects (CBP excepted). The Ptm–A relationships of these four subjects should therefore

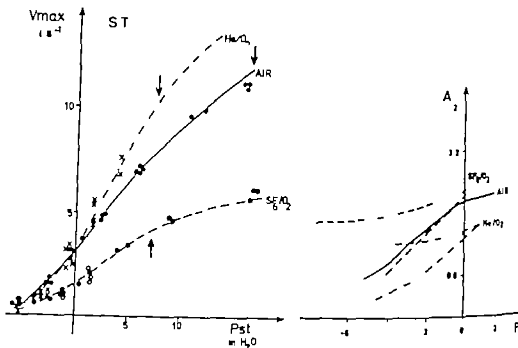


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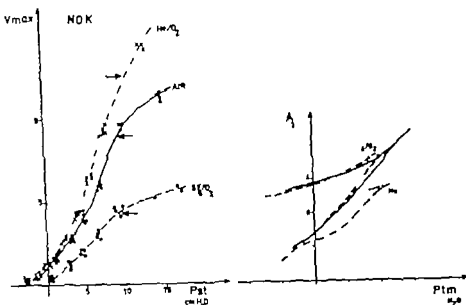


Fig. 6

...ach pressure losses would, however only affect a small part of the descending limb of the ...tm A curves presented here.

The Ptm–A relationship for the He/O mixture demonstrated consistently lower values of A for given Ptm values. In the He/O expts., laminar flow was favoured at the expense of turbulent flow and the relative contribution of viscosity dependent pressure drops was increased when switching from air to He/O_2 (Nielsen and Pedersen 1976).

At low Pst the He/O_2 expts. gave lower Vmax than the experiments with air in 4 of the subjects. Such a crossing of the Pst–Vmax curves has been observed by others (Schilder Roberts and Fry 1963), and may be due to a dominating effect of viscosity at low flow in the He/O_2 expts.

The discrepancy between the Ptm A curves for air and He/O is therefore most likely due to neglection of significant viscosity dependent pressure losses in the He/O expts. Therefore the applied model is not valid in the latter case.

The present investigation did not tell anything about density dependent pressure losses in the airway upstream from the site of flow limitation. Stenosed and kinked peripheral airways will cause A to be underestimated at given Ptm values in expirations with all three gas mixtures as shown for air in the expts. with the mechanical model (Pedersen and Nielsen 1976). Macklem and Mead (1968) measured the resistance upstream from EPP in excised human lungs during expiration of air and found that friction and convective acceleration contributed about equally to the upstream lateral pressure drop at high lung volumes, whereas friction dominated at low lung volumes. If this is the case also for the segment upstream from the flow limiting site in the intact lung, Pstr (*i.e.* the sum of viscosity and density dependent pressure losses) cannot be neglected. If Pstr varies in proportion to Vmax raised to an exponent less than 2, which would be true for diminishing dimensions of the

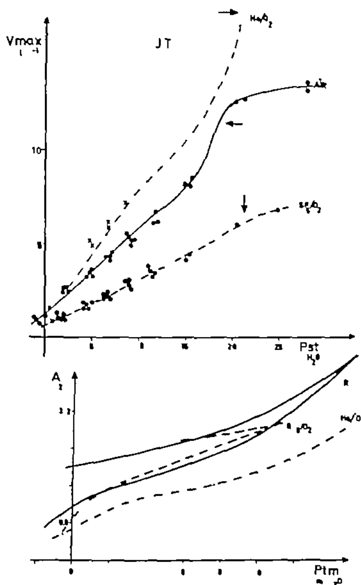


Fig. 5

be equal to the Ptm–A relationships, which we were looking for The latter term will b used in the following.

The Ptm–A curves for air and SF_6/O were almost superimposed In the SF_6/O expe turbulence was increased at the expense of laminar flow and the relative contribution o viscosity dependent pressure losses was decreased when switching from air to SF_6/O A the Ptm–A curves for the two gases were almost superimposed, we may conclude tha upstream frictional pressure losses (Pufr) (*cf* Pedersen and Nielsen 1977) if present, wer not likely to be viscosity dependent for any of the two gases (Nielsen and Pedersen 1976 and that the basic concepts of the applied model were not rejected during these circum stances.

Apparently the findings are in contrast with what has been reported by Wood and Bryan (1969) and Vibert *et al* (1971) They found that maximum expiratory flow depended less on density at long volumes below 25% VC, than above. This was attributed to increase of viscosity dependent pressure losses in the upstream part of the airway at low lung volumes.

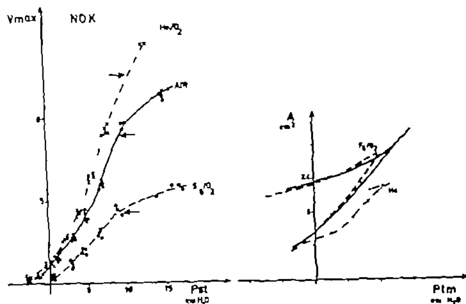


Fig. 6

Such pressure losses would, however only affect a small part of the descending limb of the P_{tm}–A curves presented here.

The P_{tm}–A relationship for the He/O_2 mixture demonstrated consistently lower values of A for given P_{tm} values. In the He/O expts., laminar flow was favoured at the expense of turbulent flow and the relative contribution of viscosity dependent pressure drops was increased when switching from air to He/O (Nielsen and Pedersen 1976).

At low P_{st} the He/O_2 expts. gave lower $\dot{V}_{max}$ than the experiments with air in 4 of the 5 subjects. Such a crossing of the P_{st} $\dot{V}_{max}$ curves has been observed by others (Schilder Roberts and Fry 1963), and may be due to a dominating effect of viscosity at low flow in the He/O_2 expts.

The discrepancy between the P_{tm}–A curves for air and He/O_2 is therefore most likely due to neglection of significant viscosity dependent pressure losses in the He/O expts. Therefore the applied model is not valid in the latter case.

The present investigation did not tell anything about density dependent pressure losses in the airway upstream from the site of flow limitation. Stenosed and kinked peripheral airways will cause A to be underestimated at given P_{tm} values in expirations with all three gas mixtures as shown for air in the expts. with the mechanical model (Pedersen and Nielsen 1976). Macklem and Mead (1968) measured the resistance upstream from EPP in excised human lungs during expiration of air and found that friction and convective acceleration contributed about equally to the upstream lateral pressure drop at high lung volumes, whereas friction dominated at low lung volumes. If this is the case also for the segment upstream from the flow limiting site in the intact lung, P_{fr} (i.e. the sum of viscosity and density dependent pressure losses) cannot be neglected. If P_{fr} varies in proportion to $\dot{V}_{max}$ raised to an exponent less than 2, which would be true for diminishing dimensions of the

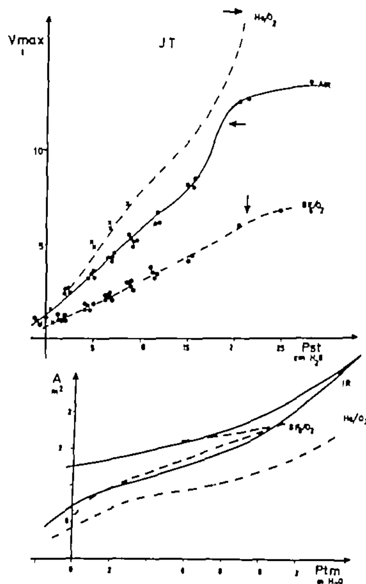


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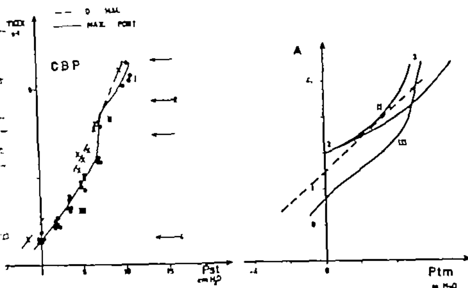


Fig. 7 Comparison between maximum effort (——) and optimal (——) curves for subject CBP. The maximum effort Ptm–A curve has a rather complicated course consisting of two descending sections (I and III) and one ascending section (II). The arabic numbers indicate the end points of these segments. The corresponding points on the Pst–Vmax curve are shown by the numbered arrows.

From the curves in Fig. 8, one cannot immediately tell which generation of bronchi will be flow limiting at a given transmural pressure. Applying the present model, however, it is possible to "translate" the Ptm–A curve for each bronchial generation into a corresponding Pst–Vmax curve (Pedersen and Nielsen 1976).

The calculated Pst–Vmax curves for generations 3, 4, 5 and 8 are shown in Fig. 9. At a given Pst the flow limiting airway generation must be the generation with the lowest Vmax (Pedersen and Nielsen 1976). During the expiration when Pst decreases, generations 3, 4 and 5 will therefore successively be flow limiting in the interval presented, corresponding almost, but not completely, to the right lower borderline of the curves in Fig. 8. In Fig. 10, each curve segment is the part of the corresponding curve in Fig. 8 actually involved in flow limitation, i.e. forming, after "translation", the lower borderline of the Pst–Vmax curves in Fig. 9. During the expiration with just maximal flow throughout, each curve segment in Fig. 10 is traversed from the right to the left, firstly the segment for generation 3. Coming to its left end, the same Pst and Vmax can now be achieved in generation 4 at larger values of both Ptm and A. But a moment later the smallest Vmax at a given Pst is provided only by generation 4. Therefore Ptm and A vary in a discontinuous manner when the site of flow limitation moves to this generation. Traversing the curve segment for generation 4, Ptm and A again decrease continuously, but when the site of flow limitation moves to generation 5, there is another discontinuous jump. Such a course of events is consistent with findings on the mechanical model (Pedersen and Nielsen 1976).

The interpretation of our findings (Fig. 3–7) with respect to which airway segments are flow limiting, is ... ted by the fact that the curves in Fig. 10 are obtained during static

airways during the expiration even in pure density dependent flow (Pedersen 1973, p. 102 f) then Ptm will be an overestimate of the true Ptm and A may be an underestimate of the true A At the same time the flow limiting segment will appear more stiff than it is (Peder and Nielsen 1976).

In the present expts. pulmonary tissue resistance was included in Pdfr but does prob not mean very much (Bachofen and Scherrer 1970)

Concluding the findings and considerations above, there is no evidence against our hy thesis that the Ptm –A curve for air (and SF_6/O_2) reflects the elastic properties of the fl limiting segments of the airway but is also influenced by upstream density dependent pre sure losses. At present, we do not know very much about such pressure losses in hum lungs. Further studies are needed to evaluate their significance.

It is only indirectly possible to get an idea of the location of the flow limiting sites in th airway at different moments during the expiration.

Because Ps is the lateral pressure drop from the alveoli to the point in the airway whe and when flow is limited, and because Ps = Pst – Ptm (*cf* Pedersen and Nielsen 1976), negative Ptm must imply that the flow limiting site is downstream from EPP which is at pressure distance equal to Pst from the alveoli. This appears to be the case during the fir part of the expiration The finding (Macklem and Wilson 1965) that EPP is in the major bronchi during the first part of the expiration, indicates that the flow limiting segment, downstream from EPP may well be the extrapulmonary airways (generation 0–ca. 3 ac cording to the terminology of Weibel (1963)) The change in sign of Ptm early in the expira tion indicates that the flow limiting segment moves upstream from EPP very soon, but whe eventually EPP moves towards the periphery as shown in dogs (Macklem and Mead 1967) it moves more rapidly than the flow limiting site and Ptm changes sign again. Such a cour of events is compatible with the findings in subjects ST JT and NOK. In subject PK, EP ultimately remained downstream from the flow limiting segment because Ptm remaind positive. In subject CBP (Fig. 7 right part, broken curve) Ptm was positive during mos of the first part of the expiration and eventually became negative, indicating roughly the same relationship between the flow limiting segment and EPP as in the 3 first subjects.

It is tempting to compare the functional Ptm –A curves with curves calculated for dif ferent airway generations *in vitro* in further search for the approximate location of the flow limiting segments in the airway

Data describing the relationship between Ptm and the ratio of the diameter D to its maxi mal size, Dmax, in airway specimens of different bronchial generations have been collected from the literature by Pardaens *et al* (1972) As $A = (D/Dmax)^2$ Amax, it is possible to calculate A at different values of Ptm, assuming Amax to be equal to the total cross-sectional area of the airway at the given bronchial generation. This cross-sectional area can be ob tained from the data of Weibel (1963) being valid for an average adult human lung at about 3/4 maximal inflation. The curves presented by Pardaens *et al* can thus be transformed to present the relationship between total cross-sectional area and transmural pressure at different levels in the bronchial system.

This is shown in Fig. 8 where the curves are shown within the interval -5 cm $H_2O <$ Ptm < 5 cm H_2O

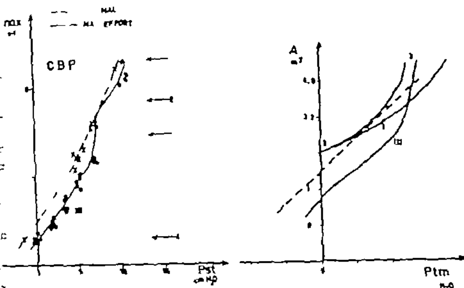


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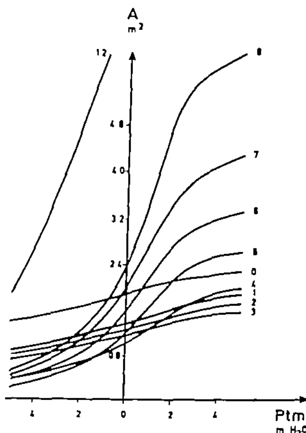


Fig. 8

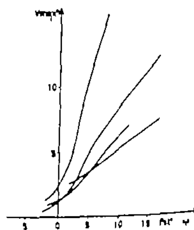


Fig. 9

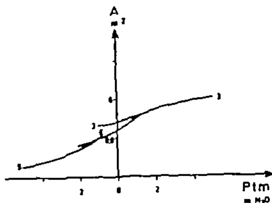
Fig. 8 Ptm–A curves calculated f r different bronchial generations using data of Pardaens *et al.* (19 and Weibel (1963). The numbers at the curves indicate the bronchial generations (trachea belongs to generation 0. A is the t tal cross-sectional area at the level of the given generation.

Fig. 9 Pst Vmax curves calculated for different bronchial levels from curves in Fig. 8 with the same numbers (*cf* text) The generation with the smallest Vmax will be flow limiti g. Therefore generations 1,4 and 5 will be flow limiting within the interval presented.

conditions and ours during dynamic. Hughes *et al.* (1974) found that the lung tissue cause the intrapulmonary airway to become less compliant in dynamic than in static conditions This would cause an upward shift of the lower portion of the curves for the intrapulmonary bronchi in Fig. 8 and make them less useful in the determination of the flow limiting site

As in the mechanical model, the ascending parts observed in most of our experimental curves may reflect shifts between different local Ptm–A curves because of upstream movement of the site of flow limitation The position of the ascending parts in the present expts. indicates that the initial sites of flow limitation may well be in the extrapulmonary airways (generations 0–ca. 3) This is seen by comparing the experimental Ptm –A curves with those in Fig. 10 Fig. 10 shows 2 shifts, firstly between generations 3 and 4 then between 4 and 5. In most our subjects, only one shift was seen This may be due to partitioning of flow between more airways coincident with peripheral movement of the flow limiting site. Small differences in the elastic behaviour of the different airways of the same generation may cause asynchronous shifts from generation to generation and smoothe the curve for the total cross-section of the airway

In the 4 subjects, who had a "closing point" in their nitrogram, the corresponding closing



g 10 Ptm–A curves for the different onchial generations connected in series, lculated from the lower borderline of e curves in Fig 9.

ressures measured on the Pst V curves (*cf* Fig. 2) were all larger than the "closing pressures obtained on extrapolation to Vmax = 0 on the Pst–Vmax curves (or to A 0 on the Ptm A curves). This was also expected as the former should indicate the Pst (not corrected for the pleural pressure gradient in vertical direction) where the most dependent airways begin to close (Dollfuss *et al* 1967) or to limit flow (Hyatt *et al.* 1973), whereas the latter is the Pst at which no more air can be expelled from the lungs. In some of the subjects, the latter was considerably negative. This raises the question whether a real negative Pst can exist without collapse of the airspaces. Provided the intraesophageal pressure is a reliable index of intrapleural pressure (*cf* review by Agostoni, 1972) our findings indicate this, and other findings *in vitro* on dog lungs (Hughes *et al.* 1970, Glaister *et al.* 1973) indicate that some airspaces remain open at considerably negative values of Pst. The airways may however remain open, because Pst is more negative or less positive than the real distending pressures across the walls of the airways (Hughes *et al* 1972, Mead *et al* 1970). In these cases the transmural pressures of the airways in static conditions need not be negative when Pst is negative.

From functional point of view the definition of the critical transmural pressure should be expanded to comprise the transparenchymal pressure. The way it is measured it is actually the sum of these two. Only if the transparenchymal pressure can be neglected we deal with a true transmural pressure. Therefore Ptm–A curves derived from MFSR curves will reflect the elastic properties of airway and lung parenchyma if the site of flow limitation is intrapulmonary and not the elastic properties of the airways alone.

By application to the lungs of the present theories there are a number of limitations, not discussed above: firstly the theories do not take into account the effect of unequal ventilation of different lung units. Secondly they do not deal with several factors associated with the collapsing airway *i.e.* airway flow resistance and inertia of the airway wall.

In the first case we will have to assume that dynamic and static recoil pressures are equal, and this is the case only if lung tissue resistance is small, and if there is no unequality of ventilation (Pedersen 1973 p. 115 ff). For normal lungs the former is probably true (Salbene and Mead 1969 Bachofen and Scherrer 1970), and the latter is probably also true due to the interdependency of airways and lung tissue (Mead *et al* 1970) causing the lung to behave in more hom on during fast expirations (Milette *et al.* 1969).

In the second case, the factors mentioned may be of importance in humans (Clém *et al* 1974 Knudson *et al* 1974) but their importance depends on the rate of change of transpulmonary pressure For these reasons airway flow is only expected to contribute to Vmax in the initial part of the expiration, due to this gradient otherwise being small. There fore peak flow may be a too large representative for alveolar flow This is especially true in patients with lung diseases (Clément *et al* 1973) but does probably not mean very much in our healthy subjects The shape of their Pst–Vmax curves does generally not indicate an initial overshoot of Vmax as evidence for airway flow

After all, the disturbing factors mentioned above may not influence the calculated area compliance curve significantly in normal subjects. Therefore the Ptm–A curve may give a real picture of the elastic behavior of the airway at the flow limiting sites at just maximum flows, especially when these sites are in the extrapulmonary airways. From a functional point of view the Ptm–A curve simply describes the elastic behaviour of a single airway which in accordance with the presented model functions in exactly the same way as the flow limiting segments of the complex system of intrathoracic airways partly imbedded in the lung parenchyma.

Appendix

The amount of gas in the lungs (TGV) is the sum of the residual volume (RV) and the volume (V) in the lungs above RV This is the case both when measured at barometric pressure (B) in the lungs, and at a different pressure (B + Palv where Palv is the alveolar pressure measured relative to the atmosphere).
We therefore have

$$TGV = V + RV \qquad A1$$

and

$$TGV_b = V_b + RV_b \qquad A$$

where the suffi es = B + Palv and b = B are the pressures at which the volumes are measured.
Using Boyle s law we get

$$TGV \cdot (B + Pal) = TGV_b B \qquad A$$

Substituting TGV from A 1 and solving for V

$$V = \frac{(TGV - RV_a)\ B - RV\ \ Pal}{B + Palv} \qquad A4$$

By use of Boyle s law

$$RV_b\ B = RV \cdot (B + P_a) \qquad A5$$

where P = Palv at the moment when V = 0, *i.e* when air stops flowing from the alveoli.
If P is small which is the case when RV is reached with most small irways open, then RV is approximately equal to RV_b, and they can both be replaced by RV
Substitution int A-4 then gives

$$V = \frac{B\ V - RV\ Pal}{B + Pal} \qquad A$$

from which the actual volume i the lungs in excess of RV can be calculated from the corresponding volume measured at barometric pressure B if the pressure in the lungs, RV and B are known.

The skilled technical assistance of Mrs Kirsten Klostergård is gratefully acknowledged.

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Acta physiol. scand. 1977 100 154–164

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Regional Blood Flow in the Left Ventricular Wall of Dogs with a Graded Coronary Artery Stenosis

By

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Received 27 August 1976

Abstract

BAGGER, H. *Regional blood flow in the left ventricular wall of dogs with a graded coron artery stenosis* Acta physiol. scand. 1977 *100* 154–164

Blood flow in the circumflex branch of the left coronary artery was recorded by electromagnetic flowmeter In the area supplied by this branch vasodilatation was produced by progressive constriction until the di stolic reactive hyperemic response to 10 s occlusion disappeared ("optimum stenosis) This degree stenosis was accompanied by a 20% decrease in diastolic circumflex flow while systolic flow remained unchanged. The distributio of blood flow in the left ventricular free wall was evaluated at optimum stenosis by counting activity i tissue blocks following bolus injection of Xe-133 into the aorta When Xe 133 was i jected immediately after occlusion of the left anterior descending branch the Xe concentration of the endocardial part of the area supplied by the circumflex branch was about half t concentratio in the epicardial part. The concentrations i the two parts did not, however differ sig ficantly when occlusion of the left anterio descending branch was omitted. This indicates that the end cardial blood flow reserve is lower tha the epicardial, and that, yet, preferential fall i blood flow the endocardial part of post-stenotic area can be rapidly eliminated when blood supply from neighbor ing artery is va lable.

Myocardial uptake of different diffusible tracers (Moir and DeBra 1967 Griggs and Nakamura 1968 Shaw Pitt and Friesinger 1972) have indicated that mean blood flows per unit weight of tissue are of the same magnitude in the epi- and endocardial parts of the left ventricular wall of the normal anaesthetized dog. This has been confirmed in some reports about the distribution of radioactive microspheres less than 15 μm in diameter (e.g. Buckberg et al 1975) Downey and Kirk (1974) have shown that systolic coronary flow supplies predominantly the epicardial half of the left ventricular wall as a consequence of the systolic intramyocardial pressure gradient. Therefore, uniform mean flows indicate that the diastolic flow must be larger and diastolic vascular resistance per unit weight of tissue smaller in the endo- than in the epicardial region. This difference in diastolic vascular resistance must be anatomical and/or functional. Cutarelli and Levy (1963) found a uniform distribution of Rb-86 in fibrillating dog hearts with intraventricular pressure equal to the normal diastolic value, normal coronary perfusion pressure and presumably ma

...n caused by a preceding period of ischemia. This result suggests that the difference in ...scular resistance between the epi- and endocardial parts of the wall is functional. However ...owney *et al.* (1975) found a 40% higher endo- than epicardial flow in fibrillating dog ...arts perfused with venous blood containing papaverine. This result seems to support the ...ea of an anatomical difference in vascular resistance.

In order to estimate the blood flow reserves the distribution of Xe-133 was measured in ...e left ventricular wall during maximum vasodilatation of the circumflex branch of the ...t coronary artery. This was obtained by progressive constriction of the branch until the ...astolic reactive hyperemic response to complete occlusion just disappeared (Reneman and ...pencer 1972, Van Der Meer 1972). The contribution of collateral flow from the left anterior ...scending branch to the area perfused by the constricted artery was evaluated by comparing ...stributions of Xe-133 obtained from dogs with and without occlusion of the left anterior ...scending branch.

Methods

...mongrel dogs of both sexes (21–60 kg) were anaesthetized with pentobarbital sodium (Nembutal®) ...mg kg. Ventilation was maintained with atmospheric air (0 275 l kg⁻ min⁻¹) through an endotracheal ...be by means of Servoventilator-900 (Siemens-Elema).

Local anaesthetic (Lidocaine-norepinephrine 1 %, 5 ml) was injected into both inguinal regions and the ...ft and right femoral arteries and veins were exposed. Ducor® "pig-tail" and left coronary artery catheters ...ere introduced through the left and right femoral arteries respectively. Under fluoroscopy the tip of the ...e pig-tail" catheter was positioned in the left ventricle. The tip of the left coronary artery catheter was ...ositioned in the aortic root just above the valve, without pointing at the region of the left coronary artery ...a Swan-Ganz 7F thermodilution catheter was introduced through the left femoral vein and the thermistor ...lodged in the pulmonary artery. The tip of polyethylene catheter was positioned in the right atrium via ...the right femoral vein. After establishing positive end expiratory pressure of 5 cm of water to prevent ...collapse of the lungs, thoracotomy was performed on the left side and the pericardium was opened. The ...furcation of the main stem of the left coronary artery was exposed by blunt dissection and loose ligature ...laced around the left anterior descending branch for subsequent occlusion. In 10 of the 12 dogs then, ...ft, ... twist copper wire was placed around the circumflex branch just distal to its origin. An electromagnetic ...low transducer and, distal to that, snare for occlusion were placed on the same branch. The flow trans...ducer was connected to Nycotron square-wave flowmeter (type 373-B).

The ECG, aortic and left ventricular diastolic pressures were recorded continuously on an oscilloscope and in two periods, immediately after positioning the catheters and after dissection of the two branches of the left coronary artery also on 4-channel ink jet recorder (Mingograf-34 Elema-Schönander). After application of the flow transducer the instantaneous flow in the left circumflex branch, the ECG and the pressures mentioned above were recorded continuously on the Mingograf 34.

The left circumflex branch was occluded for 10 and the subsequent reactive hyperemic response was recorded. I 10 dogs (group A and B) graded stenosis of the circumflex branch was produced by stepwise twisting of the surrounding copper wire with fine needle holder while stiff piece of steel wire (diameter 1 0 mm) in the loop of the copper wire was kept parallel to the circumflex branch to prevent kinking of the artery. After each twisting, the reactive hyperemic response to 10 occlusion was recorded. The stenosis ...was increased until the diastolic reactive hyperemic response to occlusion disappeared ("optimum stenosis").

Three types of experiments were performed. In group A (5 dogs) the left anterior descending branch was occluded and Xe-133 (0 9–2 7 mCi in 2.5–4 5 ml saline) was injected immediately as bolus into the aortic root. The heart was then excised in 17 (range, 13–20 s) after the injection. 20–54 min elapsed between the start of the twisting procedure and excision of the heart. In group B (5 dogs) occlusion of the left anterior descending branch was omitted. Xe-133 (2 1 2.7 mCi in 3.5 ml saline) was injected as bolus into the aortic root and the heart was excised in 17 (range 14–20 s) after the injection. In this group 31–80 min elapsed between the start of arterial constriction and excision. In group C (2 dogs) without graded stenosis ... ft circu... [illegible] 133 (1.9 mC in 2 5 ml) was injected as bolus into the aortic root ...

Acta physiol. scand. 1977 100 154–164

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Regional Blood Flow in the Left Ventricular Wall of Dogs with a Graded Coronary Artery Stenosis

By

HENNING BAGGER

Received 27 August 1976

Abstract

BAGGER, H *Regional blood flow in the left ventricular wall of dogs with a graded coronary artery stenosis* Acta physiol scand. 1977 *100* 154–164

Blood flow in the circumflex branch of the left coronary artery was recorded by electromagnetic flowmeter. In the area supplied by this branch vasodilatation was produced by progressive constriction until the diastolic reactive hyperemic response to 10 s occlusion disappeared ("optimum stenosis). This degree of stenosis was accompanied by a 20% decrease in diastolic circumflex flow while systolic flow remained unchanged. The distribution of blood flow in the left ventricular free wall was evaluated at optimum stenosis by counting activity in tissue blocks following bolus injection of Xe-133 into the aortic root. When Xe 133 was injected immediately after occlusion of the left anterior descending branch the Xe concentration of the endocardial part of the area supplied by the circumflex branch was about half the concentration in the epicardial part. The concentrations in the two parts did not, however differ significantly when occlusion of the left anterior descending branch was omitted. This indicates that the endocardial blood flow reserve is lower than the epicardial, and that, yet, a preferential fall in blood flow in the endocardial part of a post-stenotic area can be rapidly eliminated when blood supply from a neighbouring artery is available.

Myocardial uptake of different diffusible tracers (Moir and DeBra 1967 Griggs and Nakamura 1968, Shaw Pitt and Friesinger 1972) have indicated that mean blood flows per unit weight of tissue are of the same magnitude in the epi- and endocardial parts of the left ventricular wall of the normal anesthetized dog. This has been confirmed in some reports about the distribution of radioactive microspheres less than 15 μm in diameter (e.g. Buckberg et al 1975) Downey and Kirk (1974) have shown that systolic coronary flow supplies predominantly the epicardial half of the left ventricular wall as a consequence of the systolic intramyocardial pressure gradient. Therefore, uniform mean flows indicate that the diastolic flow must be larger and diastolic vascular resistance per unit weight of tissue smaller in the endo- than in the epicardial region. This difference in diastolic vascular resistance must be anatomical and/or functional Cutarelli and Levy (1963) found a uniform distribution of Rb-86 in fibrillating dog hearts with intraventricular pressure equal to the normal diastolic value normal coronary perfusion pressure and presumably maximum coronary vasodilata-

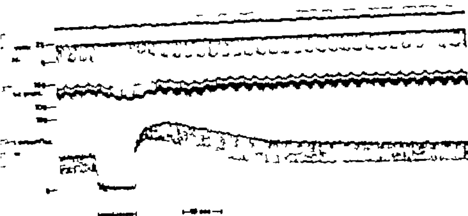


Fig. 1 The ECG, left ventricular diastolic and aortic pressures (mmHg) and the instantaneous flow in non-stenosed left circumflex coronary artery before, during and after circumflex occlusion for 10 s. The flow units are arbitrary

...creased 2 3 after the onset of occlusion. 5–10 s after reestablishment of flow these parameters had returned to their previous levels. During occlusion there was no flow in the left circumflex branch. This was followed by a period of reactive hyperemia of 60–70 s duration. Furthermore, the figure illustrates that, during hyperemia the increase in minimum systolic flow ("systolic flow") was larger than the increase in maximum diastolic flow (diastolic flow"). Fig. 2 shows recordings taken before the start of left circumflex constriction (A) at a stenosis which reduced the diastolic reactive hyperemic response (B) and finally at optimum stenosis (C). With progressive stenosis there was prolongation of the period of increased aortic pressure and increased left ventricular diastolic pressure in response to 10 s occlusion. Furthermore, diastolic flow" recorded before each occlusion ("resting diastolic flow") was reduced before optimum stenosis was obtained, while "resting systolic flow" was unchanged. In both groups of dogs "resting diastolic flow" at optimum stenosis was reduced to 80 of the value recorded before the start of constriction, while "resting systolic flow" remained unchanged. Fig. 2 also demonstrates that progressive stenosis resulted in depression of both peak diastolic and systolic reactive hyperemic flows and prolongation of the hyperemic period. At optimum stenosis the diastolic response was abolished, while a small systolic response was still present.

A representative result of the distribution of Xe 133 in the left ventricular wall is shown in Fig. 3 The isotope was injected into the aortic root immediately after occlusion of the left anterior descending branch in a dog with optimum stenosis of the left circumflex branch. The slices are numbered in the direction from the apical region towards the base of the heart. The activity concentrations of the tissue blocks of each slice are plotted in the direction from the left anterior descending branch towards the circumflex supplied area. The level of activity was very low in the area supplied by the occluded left anterior descending branch. In the area by the optimum stenosed circumflex branch the level of

mediately after occlusion of the left anterior descending branch and the heart was excised [illegible] after the injection.

After introduction of the catheters and after dissection of the left coronary artery branches oxygen, carbon dioxide tensions and the pH of arterial blood were measured as a control for respiration. Cardiac output was measured by the thermodilution method. A Wheatstone bridge and an amplifier (both manufactured in the laboratory) were used for the measurements together with a HP 130 cardiac output computer (Hewlett Packard). For each determination 10 ml of cooled saline was injected into the right [illegible] via the polyethylene catheter. Measurements were taken after introduction of the catheters, after dissection of the coronary artery branches and, in 4 of the 10 dogs, at optimum stenosis.

After excision of the heart the further procedure was exactly as previously described (Bagger [illegible]). In brief the heart was frozen in a mixture of dry ice and iso-pentane (−75°C). The apex was removed [illegible] the heart was sliced at right angles to the long axis. From each slice the left ventricular free wall [illegible] into blocks. Each block was subdivided further into epi- and endocardial halves and placed in previously weighed, cooled, closed tubes. The activity was counted in a well type scintillation detector [illegible] adjusted around the 81 keV peak and expressed as cpm/100 mg of tissue.

Results

Table I shows the hemodynamic and respiratory parameters measured in the two groups [of] dogs (A and B) immediately after introduction of the catheters (c) before the start of [the] circumflex constriction (d) and at "optimum" stenosis (s). The results of the two groups are not significantly different ($p > 0.05$)

Fig. 1 shows the ECG left ventricular diastolic and aortic pressures and the instantaneous flow in the non-stenosed left circumflex branch recorded before, during and after occlusion of the branch for 10 s. Left ventricular diastolic pressure increased and aortic pressure

TABLE I Hemodynamic and blood gas values (mean ± S.D.) in the two groups of dogs, (A) with optimum *stenosis of the left circumflex and occlusion of the left anterior descending branches* (B) optimum stenosis of the left circumflex branch alone. c Control. d After dissection of two branches of the left coronary artery. s At optimum stenosis. N = number of dogs. LVDP left ventricular diastolic pressure.

	Aortic pressure, mmHg		Heart rate beats min	Cardiac output ml min⁻¹ kg⁻	LVDP mmHg	P O_2 mmHg	P CO_2 mmHg	pH
	Systolic	Diastolic						
Optimum stenosis and occlusion of left ant. descend. branch (group A)								
c	130±16 N=5	102±13 N=5	94±21 N=5	108±26 N=5	8±5 N=5	83±8 N=5	28±6 N=5	7.42±0 N=5
d	145±11 N=5	123±12 N=5	162±15 N=5	103±26 N=4	2±8 N=5	85±12 N=5	26±5 N=5	7.41±0 N=5
s	140±9 N=5	119±9 N=5	162±9 N=5		6±9 N=5			
Optimum stenosis only (group B)								
c	136±9 N=5	106±12 N=5	103±33 N=5	141±18 N=3	7±5 N=5	93±11 N=5	27±6 N=5	7.45±0 N=5
d	137±6 N=5	111±5 N=5	137±25 N=5	144±44 N=5	5±5 N=5	96±4 N=5	29±4 N=5	7.41±0 N=5
s	141±11 N=5	116±10 N=5	138±29 N=5	118±44 N=4	2±5 N=5			

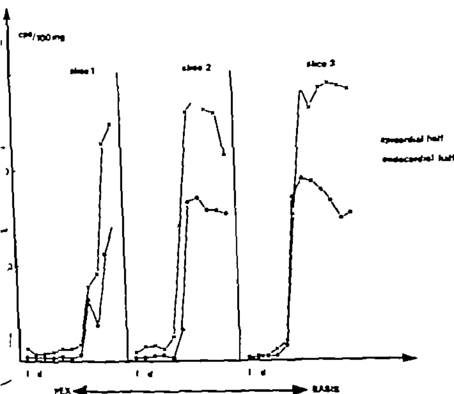


Fig. 3. A representative result of the distribution of Xe-133 in the epi- and endocardial halves of 3 slices the left ventricular wall. Xenon was injected at optimum stenosis of the left circumflex and occlusion the left anterior descending arteries. The activity of the blocks of each slice is shown in the direction om the anterior descending towards the circumflex artery

Fig. 4 is representative result of the distribution of Xe-133 in the left ventricular wall of gs with "optimum" stenosis of the circumflex branch and unimpeded flow in the left terior descending branch. In none of the five dogs of this group was a significant difference bserved between the levels of activity of the areas supplied by the left anterior descending nd circumflex branches. There was also no difference between the epi- and endocardial ctivities in these areas. Table II shows the distribution of Xe 133 in all five dogs. The mean alues of this group refer to the whole left ventricular free wall, since there was no consistent ifferences between the activities of areas anatomically supplied by the left anterior descending branch and those supplied by the circumflex.

In the two dogs with unimpeded left circumflex flow Xe-133 was injected immediately fter occlusion of the left anterior descending branch. Fig. 5 shows the results from one of hese dogs. As seen in Table II the occlusion did not appear to have any material effect on he epi- and endocardial distribution in the area of circumflex supply

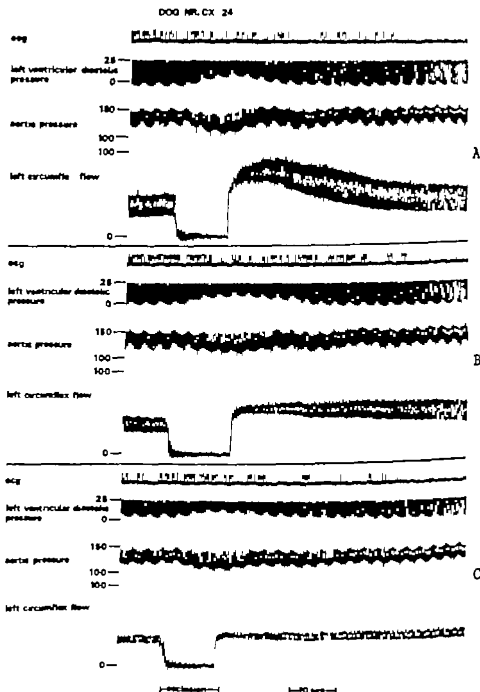


Fig. 2. The same parameters recorded as in Fig. 1 before stenosis (A) t a stenosis with decreased di hyperemic response (B) and at optimum stenosis of the left circumflex coronary artery (C).

activity was significantly higher and furthermore, was substantially higher in the epi in the endocardial region The results of all five dogs subjected to "optimum" ste of the left circumflex branch and occlusion of the left anterior descending branch (Tab show that the epicardial activity of the circumflex supplied area was about twice as hi the endocardial

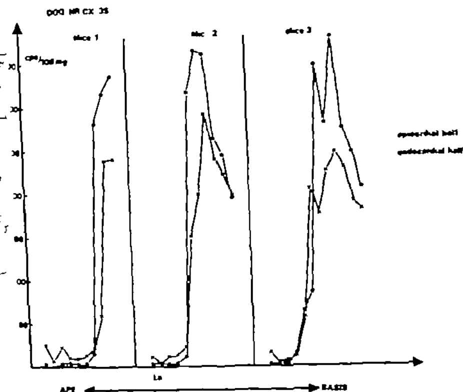


Fig. 5. One of the two results of the distribution of Xe-133 in the epi- and endocardial halves of the left ventricular wall after occlusion of the left anterior descending artery alone. The activity of the tissue blocks each slice plotted in the same way as in Fig. 3.

Discussion

After occlusion of a coronary artery mean blood flow in this artery increases temporarily ("reactive hyperemic response") indicating vasodilatation peripheral to the occlusion. Maximum peak flow is reached after occlusion for 10 s, and longer lasting occlusions only add to the response by extending the duration of the hyperemia (Khouri, Gregg and Lowensohn 1968). Reneman and Spencer (1972) and Van Der Meer (1972) used the peak diastolic reactive hyperemic flow after 10 s occlusion as an expression of the coronary blood flow reserve. This parameter will, at a given perfusion pressure and a normal diastolic intraventricular pressure, depend on the coronary vascular tonus alone. Systolic flow and therefore mean flow will also depend on systolic intramyocardial pressure. In Fig. 1 and Fig. 2 a period of decreased aortic pressure is seen after termination of the 10 s occlusion. Furthermore, a modest systolic reactive hyperemic response is still present at "optimum" stenosis of the circumflex branch. These results might indicate that the volume of tissue receiving systolic perfusion from the circumflex branch is increased immediately after reestablishment of flow. Therefore, in the present report, the peak diastolic reactive hyperemic response to

TABLE II Mean values ($\pm$ S.D) of epi- and endocardial activities and ratios of epi- to endocardial ac ($\pm$S E.) in the three groups of dogs.

	Dog no.	Number of tissue blocks	Epicard. activity $\pm$S.D cps/100 mg	Endocard. activity $\pm$S.D cps/100 mg	Epi/endo ra $\pm$S.E.
			Left ventricular circumflex area		
"Optimum" stenosis and occlusion of left ant. descend. branch	23	18	133$\pm$18	55$\pm$12	2.53 0.14
	24	17	227$\pm$68	139$\pm$48	1.79$\pm$0.18
	25	17	235$\pm$89	147$\pm$53	1.59$\pm$0. 4
	28	18	121$\pm$33	55$\pm$13	2.28$\pm$0.19
	29	17	88$\pm$21	26$\pm$6	3.38$\pm$0.19
			Left ventricular free wall		
"Optimum" stenosis only	30	34	282$\pm$47	361$\pm$76	0.80+0.03
	31	33	162$\pm$18	183$\pm$24	0.90$\pm$0.05
	32	34	740$\pm$118	712$\pm$287	1.32$\pm$0.13
	33	34	117$\pm$16	127$\pm$23	0.94$\pm$0.03
	34	34	173$\pm$17	177$\pm$29	1.00+0.03
			Left ventricular circumflex area		
Occlusion of left ant. descend. branch only	35	14	429$\pm$72	585$\pm$120	0.76$\pm$0.04
	36	16	420$\pm$55	415$\pm$47	1.03$\pm$0.06

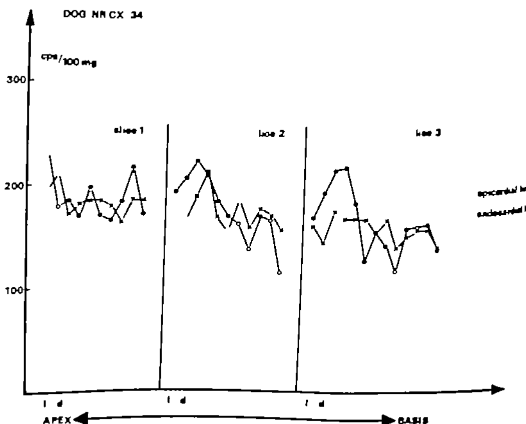


Fig. 4 A representative result of the distribution of Xe-133 in the epi- and endocardial halves of 3 sl of the left ventricular wall. Xenon was injected t optimum stenosis of the left circumflex artery T activity of the tissue blocks of each slice is plotted in the same way as in Fig.

...ory occlusion of the left coronary artery for one single systole. Therefore, it is possible that ...ong systolic compression of the endocardial vessels evokes a diastolic dilatation (which may ... characterized as a postsystolic reactive hyperemia) resulting in a decrease in the endo-...cardial blood flow reserve. It is unlikely that vasodilatation following occlusion of such ...short duration can be due to hypoxia or the liberation of anaerobic metabolites or adenosine ...(Bikens and Wilcken 1974). It might, however, result from a reflectory decrease in tone of ... vascular smooth muscles caused by the sudden change in pressure gradient across the ...vascular wall (Bayliss 1902, Sparks and Bohr 1962, Folkow 1964).

Only after occlusion of the left anterior descending branch was there a smaller flow in the ...do- than in the epicardial region of the area supplied by the "optimum" stenosed circum-...flex branch. Neither free flow in the left anterior descending branch with "optimum" stenosis ... the circumflex branch nor occlusion of the left anterior descending branch alone resulted ... endocardial hypoperfusion of the circumflex supplied area. This indicates that in 30- ...min the progressive stenosis leads to a collateral flow from the left anterior descending ...branch. This early appearance of collateral flow is in accordance with the results of Becker, ...Fortuin and Pitt (1971) and Cibulski, Lehan and Hellems (1973). Becker, Fortuin and Pitt ...(1971) and Becker, Ferreira and Thomas (1973) found that, following acute occlusion of the ...circumflex branch, collateral flow from the left anterior descending branch mainly supplied ...the epicardial region. My results indicate that collateral blood supply is also capable of ...alleviating a preferential ischemia of the endocardial region following stenosis of the artery ...anatomically supplying the area.

...his work was supported by The Danish State Research Foundation 512/3117 and The Danish Society ...for Prevention of Heart Diseases, 897

I am very grateful to Mrs. Birgit Andersen Dupont for her skilful technical assistance.

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10 s occlusion was taken as an estimate of the coronary blood flow reserve. The dis ance of this response at a certain degree of stenosis was interpreted as resulting from mum vasodilatation elicited by the stenosis.

The uniform distribution of Xe-133 in the left ventricular wall of the normal dog (1977) together with a mainly epicardial perfusion during systole (Downey a 1974) indicate that diastolic flow must be higher in the endo- than in the epicardial the wall This difference might be due to a higher degree of vasodilatation and the smaller blood flow reserve endo- than epicardially In the present study it was observe maximum coronary vasodilatation and abolished collateral blood supply that the of Xe-133 was substantially smaller in the endo- than in the epicardial half of the le cular wall This might be explained by a smaller blood flow in the endocardial pa wall The decreased diastolic flow" found at "optimum" stenosis might support planation However a decrease in the overall blood supply to the ventricular wall m lead to an increased fractional loss of xenon from vessels supplying the endocardium their passage through the epicardial part of the wall This would give rise to a ratio ured activities which is different from the ratio of flows. In the experiments with nu flow in the left anterior descending branch and optimum" stenosis of the circumfle a difference between epi and endocardial activities did not appear Therefore, it is that a diffusional loss of Xe 133 to the epicardial tissue through the wall of pre vessels supplying the endocardium should be the main cause of the difference betw and endocardial activities found in experiments with occlusion of the left anterior de branch

Either a decrease in coronary perfusion pressure to less than 70 mmHg in com with a diastolic left ventricular pressure of more than 20 mmHg (Salisbury Cr Rieben 1963) or an increase in diastolic left ventricular pressure alone up to 40 (Hirshorn and Kaber 1970) leads to subendocardial ischemia. The present expts. that decreased coronary perfusion pressure alone results in (relative) hypoperfusio endocardial half of the ventricular wall It seems unlikely that "optimum" stenosi the perfusion pressure to the critical closing pressure of the endocardial vessels. Tl stenotic pressure was not measured in the present expts. but Reneman and Spence and Van Der Meer (1972) measured a diastolic pressure of about 40 mmHg at op stenosis, while the critical closing pressure is about 15–20 mmHg according to calc by Lipscomb and Gould (1975) and Flameng *et al* (1975). In agreement with these Mosher *et al* (1964) found maximum vasodilatation at a coronary perfusion pres about 60 mmHg, while the critical closing pressure was about 25 mmHg.

In conclusion, the expts. of group A indicate that the blood flow reserve is smalle endo- than in the epicardial half of the left ventricular wall. This might be explain consequence of a higher degree of dilatation of the endocardial vessels in the norm in compensation for the smaller endo- than epicardial perfusion during systole. The endo- than epicardial blood flow reserve might explain the previously observed endo- than epicardial wash-out after traumatic, focal injections of Xe-133 in sali these regions (Andersen, Bagger and Gotzsche 1969).

Elkens and Wicken (1974) found that a reactive hyperemic response even appears

Acta physiol. scand. 1977 100 165–171

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Effects of Prostaglandins on the Isolated Human Bladder and Urethra

By

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Received 28 August 1976

Abstract

…dersson, K.-E., A. Ek and C. G. A. Persson. *Effects of prostaglandins on the isolated human bladder and urethra.* Acta physiol. scand. 1977. *100.* 165–171

…e effects of prostaglandins $F_{2\alpha}$ ($PGF_{2\alpha}$), E_1 (PGE_1) and E_2 (PGE_2) on the human lower urinary tract were …died in vitro in preparations obtained from patients undergoing total cystourethrectomy because of …adder malignancy. Tissue specimens were taken from different parts of the urethra, the urethrovesical …ction, and the bladder. From these specimens, smooth muscle preparations …ere dissected and mounted …organ baths, that were filled with Krebs solution (37°C) and bubbled with carbogen. Isometric tension … recorded. Preparations from the bladder and all parts of the urethra were contracted by $PGF_{2\alpha}$. This …fect was not affected by tetrodotoxin, phenoxybenzamine, or atropine; isoprenaline relaxed the $PGF_{2\alpha}$ …duced contractions. PGE_1 and PGE_2 both contracted strips from the bladder. However, urethral prepara…ns contracted by $PGF_{2\alpha}$ or noradrenaline were relaxed by these agents. The relaxing effect …as at least pronounced as that produced by isoprenaline; it was not affected by propranolol.

…udies on isolated smooth muscle preparations of the lower urinary tract in man have …own that the bladder and the bladder neck contract in response to acetylcholine (Todd …d Mack 1969, Nergårdh and Boréus 1972, Awad *et al.* 1974, Caine *et al.* 1975, Ek *et al.* …976). Noradrenaline relaxes the bladder, but contracts the bladder neck (Awad *et al.* 1974, …undin *et al.* 1976). Relaxation-mediating β-adrenoceptors have been demonstrated in the …adder neck (Awad *et al.* 1974, Caine *et al.* 1975) and the proximal urethra (Ek *et al.* 1976), …ut the dominating response of these structures to both acetylcholine and noradrenaline is a …ontraction (Awad *et al.* 1974, Caine *et al.* 1975, Ek *et al.* 1976). No endogenous agent …eems to be known which can relax the smooth muscle of the bladder-urethral junction.

In experiments on cats, Persson (1976) found that the prostaglandins E_1 and E_2 relaxed …e isolated, perfused bladder-urethral junction. To test whether this effect could be demon…strated also in the human lower urinary tract, the present expts. were performed. The actions

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Abstract

...dersson, K. E., A. Ek and C. G. A. Persson. *Effects of prostaglandins on the isolated human bladder and urethra.* Acta physiol. scand. 1977. *100.* 165–171

...of prostaglandins $F_{2\alpha}$ ($PGF_{2\alpha}$), E_1 (PGE_1) and E_2 (PGE_2) on the human lower urinary tract were ...ied in vitro in preparations obtained from patients undergoing total cystourethrectomy because of ...adder malignancy. Tissue specimens were taken from different parts of the urethra, the urethrovesical ...ction, and the bladder. From these specimens, smooth muscle preparations were dissected and mounted ... organ baths, that were filled with Krebs solution (37°C) and bubbled with carbogen. Isometric tension ...s recorded. Preparations from the bladder and all parts of the urethra were contracted by $PGF_{2\alpha}$. This ...fect was not affected by tetrodotoxin, phenoxybenzamine, or atropine; isoprenaline relaxed the $PGF_{2\alpha}$ ...duced contractions. PGE_1 and PGE_2 both contracted strips from the bladder. However, urethral prepara...ons contracted by $PGF_{2\alpha}$ or noradrenaline were relaxed by these agents. This relaxing effect was at least ...pronounced as that produced by isoprenaline; it was not affected by propranolol.

...tudies on isolated smooth muscle preparations of the lower urinary tract in man have ...hown that the bladder and the bladder neck contract in response to acetylcholine (Todd ...nd Mack 1969, Nergårdh and Boréus 1972, Awad *et al.* 1974, Caine *et al.* 1975, Ek *et al.* 1976). Noradrenaline relaxes the bladder but contracts the bladder neck (Awad *et al.* 1974, ...ndin *et al.* 1976). Relaxation-mediating β-adrenoceptors have been demonstrated in the ...ladder neck (Awad *et al.* 1974, Caine *et al.* 1975) and the proximal urethra (Ek *et al.* 1976), ...ut the dominating response of these structures to both acetylcholine and noradrenaline is a ...ontraction (Awad *et al.* 1974, Caine *et al.* 1975, Ek *et al.* 1976). No endogenous agent ...eems to be known which can relax the smooth muscle of the bladder-urethral junction.

In experiments on cats, Persson (1976) found that the prostaglandins E_1 and E_2 relaxed the isolated, perfused bladder-urethral junction. To test whether this effect could be demonstrated also in the human lower urinary tract, the present expts. were performed. The actions

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of prostaglandins $F_{2\alpha}$, E and E_2 on isolated preparations from the urethra, the urethrova… junction and bladder obtained from patients undergoing total cystourethrectomy … investigated.

Material and Methods

Tissue specimens

Pieces of tissue from the urethra, the urethrovesical junction, and the bladder were obtained from … (50–66 years) and two women (64 and 74 years) undergoing total cystourethrectomy because of … of the bladder Pre-operative radiological treatment (4500 rad) had been given to 6 of the male and female patients.

Immediately after removal of the bladder and the urethra, tissue specimens were taken from … parts of the urethra They were placed in chilled Krebs solution. The sites where the tissue pieces … were carefully noted on a schematic drawing of the lower urinary tract. As this was done for each … it was possible to select the specimens so that preparations from the whole length of the urethra … investigated.

Tissue specimens were obtained from 2 to 4 different levels of the urethra of each patient 6 prep… were dissected from tissue specimens taken from the urethrovesical junction longitudinally in the … of the urethra, or transversely anteriorly or posteriorly From the 3 non-irradiated male patients, … one of the women, specimens were also taken from the lateral walls of the bladder care being … avoid tissue that was macroscopically abnormal

Preparation and mounting

As soon as possible, the tissue specimens were transported from the operating-room to the lab… From each piece of tissue, smooth muscle preparations were dissected The urethral preparatio… mounted either as tubal segments, leaving the mucosa i tact or as strips of tissue. The tubal … which were approximately 2 3 mm long, were mounted o two thin steel rods, one being fixed, … other connected to a f rce transducer (Grass Ft03). The strip preparations were obtained by cutt… a tubal segment and removing most of the mucosa. Thus, both types of urethral preparation all… recording mainly of circular muscle activity as isometric tension changes. From the urethrovesical … and the bladder walls, preparations were dissected as tissue trips (removing most of the mucosa) appro… mately 2–4 mm wide, 2 3 mm thick, and 15–20 mm long. All recordi gs were done by means of Gr… polygraph (model P7).

W thin 1 h after removal of the bladder and the urethra, the isolated preparations were dissected … mounted in jacketed, thermostatically controlled organ baths (30 ml), filled with Krebs solution that … bubbled with 95% O_2 nd 5% CO_2. The Krebs solution had the following composition (mM): NaCl … KCl 4 6, $CaCl_2$ 2.5 MgSO 1 15 $NaHCO_3$ 24 9 KHPO 1 15 glucose 5.5 pH 7 40.

After mounting, the initial tension of the preparat ons was set at 0.5 g (bout 0.7 mN/mm²) Dur… an initial period of equilibratio (bout 1 h) the basal tension was adjusted so that the preparation rea… a stable tension level of bout 0.3 g

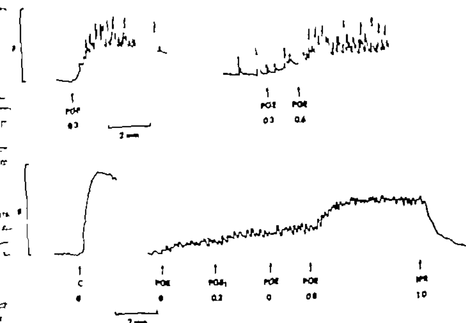
A t tal of 32 preparations from the urethra and the urethrovesical junction, and 12 preparations from … bladder were studied. 4 of the urethral, and 2 of the bladder preparations were not responsive t drugs … were discarded. A few preparations were tored overnight at 4°C. They responded to drugs as did … preparations investigated on the day of the operation. N differences in responses were found betw… preparations from irradiated and non-irradiated patients.

Drugs

Drugs used were (−) noradrenaline bitartrate, (±) adrenaline bitartrate, (±) isoprenaline hydrochlo… (Sigma Chemical Compa y USA), propranolol hydrochloride (ICI Ltd, U K.), phenoxybenzamine hy… chloride (Smith, Kl ne and French, U K.), acetylcholine chloride (Calbiochem, USA) tropine sulp… (Pharmacopea Nordica), prostaglandin $F_{2\alpha}$, prostaglandin E_1 prostaglandin E_2 (AB ASTRA, Swe… tetrodotoxin (Sankyo, Japan). The drugs were injected directly into the bath. Concentrations give… final bath concentrations.

Results

21 of the 28 responsive preparations from the urethra, and 8 of the 10 responsive blad… strips exhibited a spontaneous contractile activity that often … or disappea…



g. 1. Upper panel: contracting effects of $PGF_{2\alpha}$ and PGE_2 on an isolated strip from the lateral part of the human detrusor. Lower panel: contracting effect of acetylcholine (ACh) and PGE_1 on an isolated strip from the lateral part of the human detrusor. The contraction induced by PGE_1 is relaxed by isoprenaline (IPR). Concentrations are given in μg/ml.

uring the course of an expt. The activity was unaffected by tetrodotoxin (0.5 μg/ml), atropine (0.1–0.3 μg/ml), and phenoxybenzamine (0.1 μg/ml). However, it could be inhibited by isoprenaline (0.1–1.0 μg/ml).

Effects of prostaglandins on the bladder. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 0.05–1.0 μg/ml, prostaglandin E_1 (PGE_1), 0.2–2.0 μg/ml, and prostaglandin E_2 (PGE_2), 0.2–2.0 μg/ml, contracted the isolated bladder preparations in a concentration-dependent way (Fig. 1). The sensitivity to the prostaglandins varied between the preparations, and tended to increase during an expt. $PGF_{2\alpha}$ was consistently found to be more potent and to produce contractions of higher amplitude than PGE_1 and PGE_2; potency relations were difficult to calculate because of the wide variation in sensitivity between the preparations and because of the small number of preparations that were investigated. The contractions induced by the prostaglandins developed slower than those caused by acetylcholine, 0.05–0.5 μg/ml, and were often characterized by rhythmic changes in tension (Fig. 1). The responses to the prostaglandins were unaffected by tetrodotoxin, 0.5 μg/ml, phenoxybenzamine, 0.1 μg/ml, and atropine, 0.3 μg/ml, but were counteracted by isoprenaline, 0.1–1.0 μg/ml (Fig. 1).

Effects of prostaglandins on the urethra. $PGF_{2\alpha}$ (0.05–1.0 μg/ml) had a concentration-dependent contracting effect on all parts of the isolated urethra, including the urethrovesical junction (Fig. 2). The sensitivity to the prostaglandin varied between different preparations taken from the same urethra, but the results did not suggest that any part of the urethra was

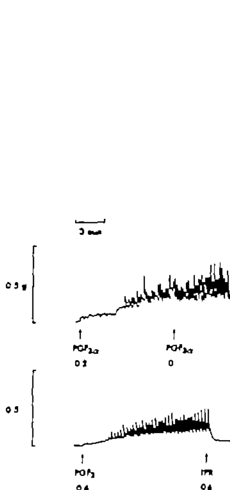


Fig. 2

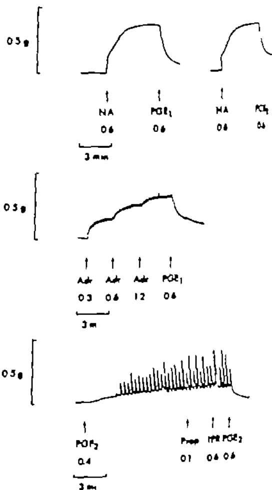


Fig. 3

Fig. 2. Contracting effects of $PGF_{2\alpha}$ on two different strips of the human urethra taken from the distal part of the prostatic urethra. The contractions are effectively relaxed by isoprenaline (IPR). Concentrations are given in μg/ml.

Fig. 3. Relaxing effects of PGE_1 and PGE_2 in urethral preparations contracted by noradrenaline (NA), adrenaline (Ad.), and $PGF_{2\alpha}$. Propranolol (Prop) had no influence on the $PGF_{2\alpha}$-induced response. However, propranolol blocked the relaxing effect of isoprenaline (IPR), but not that of PGE_2 (lower tracing). Preparations taken from the proximal part of the prostatic urethra (upper tracings), the bulbous urethra (middle tracing), and the distal part of the prostatic urethra (lower tracing). Concentrations are given in μg/ml.

particularly sensitive to $PGF_{2\alpha}$. Similar to its effects on the bladder, the actions of $PGF_{2\alpha}$ on the urethra were characterized by a slowly developing contracture with superimposed rapid changes in tension (Fig. 2). The contractions were not influenced by tetrodotoxin, 0.5 μg/ml, phenoxybenzamine 0.1 μg/ml or atropine, 0.3 μg/ml. However, a $PGF_{2\alpha}$-contracted preparation was effectively relaxed by isoprenaline, 0.1–1.0 μg/ml (Fig. 2).

PGE_1, 0.1–1.0 μg/ml and PGE_2, 0.1–1.0 μg/ml, decreased the contractions of spontaneously active preparations and lowered the basal tension. Both E prostaglandins relaxed preparations contracted by noradrenaline, 0.3–1.0 μg/ml, adrenaline, 0.3–1.3 μg/ml, and $PGF_{2\alpha}$ (Fig. 3). The relaxing effect was concentration-dependent and as rapid in onset

produced by isoprenaline. The maximum effect that could be obtained was often greater that caused by isoprenaline. Thus a preparation maximally relaxed by this amine d be further relaxed by the prostaglandins. Within the concentration-range used, no rence in potency between PGE and PGE_2 was found. The relaxing effect resisted tment with tetrodotoxin, 0.5 µg/ml, and propranolol, 0.1 µg/ml. However propranolol is concentration prevented the effects of isoprenaline, 0.1 1.0 µg/ml.

Discussion

mal expts. have shown that prostaglandins both of the E and F series contract the urinary dder. Thus, Namzada (1967) showed that PGE increased the tone and motility of the nea-pig and rat bladder *in situ*, but failed to do this *in vitro*. Ambache and Zar (1970) nd that $PGF_{2\alpha}$ and PGE_2 produced sluggish, long-lasting contractions of the isolated nea-pig bladder. They found that the sensitivity of the preparations was low and that $F_{2\alpha}$ had a weaker effect than PGE_2. In dog urinary bladder Taira (1974) found that a gle injection of $PGF_{2\alpha}$ into the caudal vesical arteries produced a slowly developing tained increase in bladder tone. Abrams and Feneley (1976) showed that isolated strips the human urinary bladder were contracted by $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE and PGE_2. Among se agents, $PGF_{2\alpha}$ was the most potent, PGE the least. Compared with the contractions luced by acetylcholine, the responses to the prostaglandins were slower and had a relaxa- a phase lasting up to 20 min. This was confirmed in the present study. It was further own that the responses to $PGF_{2\alpha}$, PGE_1 and PGE_2 were unaffected by tetrodotoxin. This in agreement with the findings of Taira (1974) in the dog bladder and seems to rule out ural excitation as a part of the response. The possibility that the prostaglandins might act rough adrenergic or cholinergic transmitter released by a mechanism independent of ural excitation seems unlikely for several reasons. The response of the normal human trusor to noradrenaline is relaxation (Awad *et al.* 1974; Sundin *et al.* 1976) and α-adreno- ptors did not seem to be involved in the prostaglandin-induced contractions, as phenoxy- nzamine had no effect on the responses. Also atropine was without effect. This was not rprising, as it is well known that bladder contractions in response to parasympathetic mulation are quite resistant to atropine. However Taira (1974) obtained evidence that e actions of $PGF_{2\alpha}$ in the dog bladder did not involve a cholinergic mechanism, as the sponses were not modified by doses of physostigmine which greatly augmented bladder ontractions to pelvic nerve stimulation and to acetylcholine. Taken together these results ggest that the prostaglandins act directly on the bladder musculature.

Data on the effects of prostaglandins on the urethra are scarce. In recent study on the olated, perfused urethrovesical junction of cats (Persson 1976), it was found that prosta- landin $F_{2\alpha}$ contracted the preparation and increased the resistance to flow. PGE_1 and PGE_2, n the other hand, had relaxing effects. The present results showed that the prostaglandins ad similar effects on the isolated human urethra, including the urethrovesical junction. $PGF_{2\alpha}$ induced slowly developing contractions that were unaffected by tetrodotoxin, phen- oxybenzamine, and atropine. Isoprenaline effectively counteracted these contractions. In reparations contracted by $PGF_{2\alpha}$ or noradrenaline, PGE_1 and PGE_2 had a relaxing effect

that was as rapid in onset and at least as pronounced as that produced by isoprenaline. mechanism for this relaxing effect did not seem to involve β-adrenoceptors, as the resp was present also after blockade of these receptors by propranolol.

The responses to PGE and PGE_{2}, *i e* contraction of the bladder and relaxation of the urethra, seem to be functionally appropriate for bladder emptying. However the contrac responses of the bladder to the prostaglandins developed relatively slowly compared to that produced by acetylcholine. This makes it less likely that the prostaglandins play a r for the detrusor contraction causing the evacuation of the bladder but does not excl that they might be of importance for *e.g* maintaining tone in this organ. Gilmore and Vu (1971) suggested that release of prostaglandins might be a local mechanism designed accommodate the urinary bladder to filling. Hills (1976) found that the spontaneous act and tone in isolated strips of detrusor and trigone muscle from various species, includ man was reduced by indomethacin, meclofenamic acid, and flufenamic acid, all inhibit of prostaglandin synthesis and/or action Involvement of prostaglandins in bladder co traction was suggested by Johns and Paton (1976) They found that indomethacin reduc the residual response elicited by transmural stimulation of rabbit detrusor muscle in t presence of atropine.

Concerning relaxation of the lower urinary tract during evacuation of the bladder, t interest has mainly been focused on adrenergic mechanisms. The involvement of β-adren ceptors in the relaxation of the bladder outlet was proposed by Nergårdh (1974). In ex on the isolated, perfused bladder-urethral junction of the cat he found that adrenaline a noradrenaline in low concentrations reduced the resistance to flow However Persson a Andersson (1976) using a similar model, were unable to confirm these results, finding t the amines, even in low concentrations, consistently increased the resistance to flow

Jonas and Tanagho (1975) showed that in dogs where the body of the bladder was su cally separated from the urethra, detrusor contraction induced a drop in the urethral re ance They contended this to be caused by a nerve-mediated spinal reflex. Ghoneim et (1975) using a similar experimental model confirmed these findings, and showed that changes involved both the plain and striated muscles of the urethra. They proposed t inhibitory β-adrenergic mechanisms were partially responsible for the responses. Howe they could not exclude that other complicated mechanisms were involved in the change

Both acetylcholine and noradrenaline contract the human urinary bladder outlet (N gårdh and Boréus 1972, Awad *et al* 1974 Caine *et al* 1975 Ek *et al* 1976) As the uret wall tension at rest partially seems to be determined by the sympathetic activity (Awad e 1976), a decrease in urethral resistance can be produced by blocking the α-adrenocep and/or by decreasing the sympathetic tone. So far no endogenous agent has been dem strated that per se can relax the bladder-urethral junction and the urethra. However present findings suggest that PGE and PGE are able to produce this effect.

This investigation was supported by grants from the Swedish Medical Research Council (project 17X-04264).

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Acta physiol. scand. 1977 100 172–181

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Bronchial Effects of Some Prostaglandin E and F Analogues

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Received 26 October 1976

Abstract

STRANDBERG K and P HEDQVIST *Bronchial effects of some prostaglandin E and F an*
Acta physiol scand. 1977 *100* 172–181

The effects of some nalogues f PGE_1, PGE_2 and $PGF_{2\alpha}$ rendered less accessible to the actio staglandin dehydrogenase by having methyl groups at carbons 15 and/or 16, or a phenyl group 17 on the tracheobronchial insufflation pressure in guinea pigs and on the tone of isolated human were investigated Regardless of rout of administration, i v i jection or aerosol administratio 16 16-dimethyl PGE PGE₂, the methyl esters of 15(R)15-methyl PGE_2 and 15(S)15-meth 16 16-dimethyl-PGE_2 and its methyl ester and 17-phenyl PGE_2 all produced a dose-dependent i of the histamine induced increase i insufflation pressure and a fall in systemic blood press nesthetized guinea pig. Although the durati n of action of the PGE-analogues was longer the the parent compounds, none of them was more potent with regard t the peak effect produced o tion pressure. Of the $PGF_{2\alpha}$ analogues studied, 16,16-dimethyl $PGF_{2\alpha}$ was the most potent com decreasing ra k order followed by 15(S)15-methyl $PGF_{2\alpha}$, 17 phenyl-$PGF_{2\alpha}$ and $PGF_{2\alpha}$, in th increase i insufflation pressure on i i jection. O aerosol administration, only 16,16-dimeth compound was more potent than $PGF_{2\alpha}$ On isolated human bronchi, PGE_1 and PGE_2 were co relaxant, although in some expts the relation t PGE_2 was f llowed by constriction. The 16,16 nalogues of PGE nd PGE_2 were consistently bronchoconstrictor whereas the effects of the ot analogues were weak d nconsistent. In compariso with the bronchorelaxant β_2-adrenoceptor st compounds, the PGEs nd PGE-analogues studied d not have any outstanding features and imply a risk in clinical practice.

In general, prostaglandins (PGs) of the E series cause bronchodilatation whereas the F series constrict bronchi (cf Smith 1972) Upon *in vivo* administration these rapidly degraded into biologically less active compounds, principally by the action of glandin dehydrogenase (PGDH) (Änggård and Samuelsson 1967). The lung has capacity in this respect as demonstrated by the finding that more than 90 per cen injected PGE or PGF is metabolized by one single passage through the lung cir (Ferreira and Vane 1967) The short half life of the primary PGs consequently lim clinical usefulness. Therefore, several PG-analogues have been synthesized, in w 15-hydroxy group is made less accessible to the action of PGDH These agents, not 15-methyl and 16,16-dimethyl analogues of PGE_2 and $PGF_{2\alpha}$, have proved more pot

-acting than their parent compounds as stimulators of uterine contractions and as -itors of gastric secretion (Wiqvist *et al.* 1973, Karim *et al.* 1973, Robert 1976, Nylander Andersson 1976, Karim and Fung 1976).

-he present investigation was undertaken to probe further into the action of PGs on the -heo-bronchial tree. Experiments were carried out to determine the effects of some ana- -es of PGE, PGE$_2$ and PGF$_{2\alpha}$, rendered less accessible to the action of PGDH by having -yl groups at carbons 15 or 16, or a phenyl group at carbon 17, on the tracheobronchial -nflation pressure in guinea pigs and on the tone of isolated human bronchi.

Methods

-ea pigs

-albino guinea pigs, weighing 500–800 g, were anaesthetized with sodium pentobarbitone (60 mg/kg) -with supplements when necessary. The animals were prepared for recording of tracheal insufflation -sure, as described in detail by Strandberg and Hedqvist (1975). The animals were ventilated with -ar constant volume respirator (42 strokes/min, 4–6 ml/kg), and changes in the insufflation pressure -measured with Statham pressure transducer (P23 BC) connected to side-arm of the tracheal -ula. The systemic arterial blood pressure and the heart rate were monitored from the right carotid -ry using Statham pressure transducer (P23 AC). All parameters were recorded on Grass polygraph. -he beginning of the expts. heparin (500 IU/kg) was given i into jugular vein. Drugs were administer-d either by this route or as aerosols (2 min) via the air inlet channel of the ventilator to which was -ected De Vilbiss nebulizer Model 35 A, nebulizing 0 2 ml/min of the fluid instilled (5 ml). Care was -n to minimize condensation of water by keeping the air passages warm by lamps. The aerosol reached -tracheal cannula within 5

-he bronchoconstrictor activity of administered compounds was expressed as the percentage increase -racheal insufflation pressure produced at peak height of the response. The bronchodilator activity was -ssed in an indirect way. A dose of histamine producing approximately 200 per cent increase in insuffla- -n pressure was given After recovery the compound to be tested was administered or as aerosol -min) followed respectively 30 and 2 min later by the test dose of histamine The bronchodilator -vity was then calculated from the difference in histamine responses and was expressed as per cent inhibi- -n of the control response to histamine.

-man bronchi

-ng specimens were obtained from 11 patients, of either sex, undergoing pulmonary surgery. The tissues -re transported to the laboratory in cooled Tyrode solution and were either used for immediate expts. -stored overnight at +4°C. In our experience such storage does not impair the reactivity of the bronchial -ooth muscle to broncho-active agents. Helical strips, from free-dissected macroscopically normal bronchi, -re mounted in 5 ml organ baths containing Tyrode solution kept at 37°C and gassed with 5% CO_2 in -. The composition of the Tyrode was (concentrations in mM): NaCl 136 7, KCl 2.7, $CaCl_2$ 1 8, $MgCl_2$ -, $NaHCO_3$ 11 9, NaH_2PO_4 0.4, glucose 5.5. Basal tone of the preparation and changes of the contractile -on were measured isometrically with force displacement transducer (FT 03) and were recorded on -rass polygraph. Tension equivalent to the load of 0.5 g was applied, and the strips were left for 1 h of -quilibration. Thereafter when required, the tension was adjusted to the original level, and cumulative -se-response curves for histamine and isoprenaline were determined. Increasing doses of either compound -as administered to the bath every two min until maximal responses were obtained. By this procedure -ontraction-relaxation span was established for each preparation. After washing the preparation, hista- -ine was administered until the response was approximately 50 per cent of the above-mentioned span. -hen, cumulative dose-response curves for primary PG and one or several of its analogues were deter- -ined. Starting the determinations from the middle of the contraction-relaxation span allowed assess- -ment of either constrictor or relaxant properties of the compounds. All expts were terminated by repeating -the administration of isoprenaline.

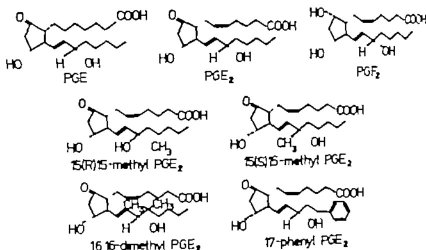


Fig. 1 Principal structures of PGs and PG-analogues used in the study

Drugs

The following drugs were used PGE 16,16-dimethyl-PGE PGE_2, the methyl esters of 15(S)15-me- PGE_2 and 15(R)15-methyl-PGE_2, 16,16-dimethyl PGE_2 and its methyl ester 17-phenyl-PGE_2, PG 15(S)15-methyl-$PGF_{2\alpha}$ and its methyl ester 16,16-dimethyl $PGF_{2\alpha}$, 17-phenyl-$PGF_{2\alpha}$, histamine h chloride and isoprenaline sulphate. The prostaglandins, kindly supplied by Upjohn Co Kalam Michigan, U.S.A were dissolved in ethanol (10 mg/ml) and kept at −20°C until use. Immediately be each expt. aliquots were evaporated to small volume by a stream of itrogen and made to appro volumes by the addition of saline. The principal structures of the PGs are shown in Fig 1 Histamine isoprenaline were of reagent grade from rdinary commercial sources, and the amounts quoted expressed in terms of the base.

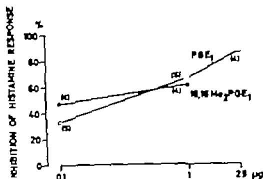
Results

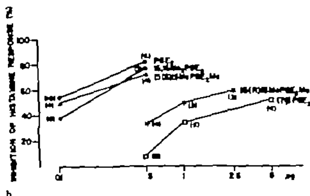
Effects of PGE-analogues

I v administration of PGE or PGE_2 or their analogues 16,16-dimethyl-PGE 16 dimethyl-PGE_2, 15(S)15-methyl-PGE_2, 15(R)15-methyl-PGE or 17 phenyl-PGE 30 s p to I.v injection of histamine consistently counteracted the histamine-induced increa insufflation pressure in the guinea pig (Fig. 2) On i.v injection 16,16-dimethyl-PG appeared equipotent to PGE in this respect. The PGE_2-analogues were either equipo to or less active than the parent compound. The duration of action of the analogues w however always much longer than that of the parent compounds (Fig. 3) Essentially sim effects were obtained on aerosol administration of the PGEs and their analogues (Table

PGE_1, PGE_2, and their analogues produced a fall in systemic arterial blood press The quantitative aspects of this effect were not investigated.

Addition of histamine (0.2–16 µg/ml) to isolated human bronchi consistently resulted constriction, whereas isoprenaline (0 05–0.3 µg/ml) and PGE (0.2–30 µg/ml) always ca relaxation (Fig. 4). The effect of PGE_2 (0.2–6.4 µg/ml) on bronchial tone was more varia In 5 out of 11 tested strips PGE_2 caused a sustained, moderate relaxation (Fig. 5). In remaining 6 expts. it caused an initial relaxation followed by a prolonged moderate const

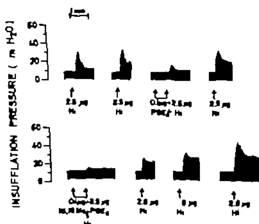




b

2 Percentage inhibition by [...]jected PGE_1 and PGE_1 analogues (A), and PGE_2 and PGE_2 analogues (B), on histamine-induced increase in tracheal insufflation pressure in artificially ventilated guinea pigs. Histamine [...] 30 [...] after PGs and by the [...] route. Shown are means from 3–10 expts.

On equimolar basis the relaxant effect of PGE_1 and PGE_2 was always weak compared [t]hat of isoprenaline (Fig. 4 and 5). 16,16-dimethyl-PGE_2, administrated over a wide range [of d]oses (0.01–0.16 µg/ml), either as free acid or as methyl ester did not relax any of 11 [...]ed strips but rather proved a consistent and potent broncho-constrictor compound [...] 5). The corresponding analogue of PGE 16,16-dimethyl-PGE_1 either caused no effect



[...] 3. Time course of inhibition of histamine-induced increase in tracheo-bronchial insufflation pressure by i[...] administration of PGE_2 16,16-dimethyl-PGE_2 methylester in the [guinea] pig. Time interval between administration of histamine was 10 min.

TABLE I Percentage inhibition by aerosols of PGE_1, PGE_2, and PGE analogues of his increase in tracheal insufflation pressure in artificially ventilated guinea pigs. Mean valu from 3–7 expts. are given.

Drug	Dose μg/ml	Number of expts.	% Inhibition means ± S.D
PGE	1.0	5	37 ± 17
	5.0	4	67 ± 6
$16,16Me_2PGE_1$	1.0	5	61 ± 29
	0.025	7	22 ± 10
PGE_2	0.1	7	58 ± 17
	1 0	5	63 ± 12
$16\ 16Me_2PGE_2$	0 1	3	47 ± 38
	1 0	7	61 ± 13
$15(S)15MePGE_2$	0 1	4	29 ± 29
	1 0	7	40 ± 19
$17\text{-phenyl-}PGE_2$	5 0	5	15 ± 18

or a slight contraction when tested on 5 strips (Fig. 5) The effects of the other analogu PGE_2 were weak and inconsistent Thus, 15(R)15-methyl PGE_2 methyl ester (0 02-41 ml) relaxed 2 strips (*cf* Fig. 5) and had no effect whatsoever on 5 preparations, whe 15(S)15-methyl PGE_2 methyl ester (0.2–3.2 μg/ml) produced no effect on 2 strips and c striction on 6 strips. *Finally 17 phenyl-PGE_2 (0.2–12.8 μg/ml)* relaxed 2 strips and ha effect in 3 preparations.

$PGF_{2\alpha}$-analogues

On i.v injection in guinea pigs $PGF_{2\alpha}$ and its analogues consistently produced an inc in tracheal insufflation pressure (Fig. 6) The dose-response curves for these compo compared to that of histamine are given in Fig. 7 On a weight basis the rank order o tency for the compounds was Histamine > 16,16-dimethyl-$PGF_{2\alpha}$ > 15(S)15-methyl-PG methyl ester > 15(S)15-methyl-$PGF_{2\alpha}$ > 17 phenyl-$PGF_{2\alpha}$ > $PGF_{2\alpha}$. At equiactive doses

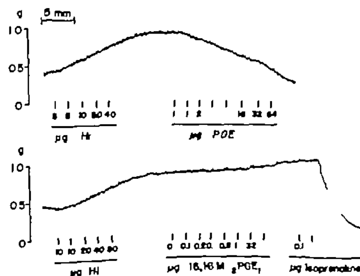
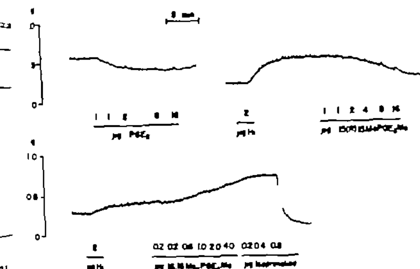


Fig. 4 Cumulative dose-eff lations for histamine, PGE, dimethyl-PGE and isopre the tone of an isolated b bronchus incubated in Tyrode



5 Cumulative dose-effect relations for PGE2, 15(R)15-methyl-PGE2 methylester 16,16-dimethyl-PGE2 methylester and isoprenaline on the tone of an isolated human bronchus incubated in 5 ml Tyrode. Single concentrations of histamine.

...ation of the response to $PGF_{2\alpha}$ was considerably shorter than that to its analogues. ...th the doses used the latency to reach the peak effect was consistently shorter for $PGF_{2\alpha}$ ...n for 15(S)15-methyl-$PGF_{2\alpha}$ and 16,16-dimethyl-$PGF_{2\alpha}$. These two compounds either ...roduced a slow progressive increase in insufflation pressure or a "two peak effect" where ...e second peak constituted the major increase (Fig. 6). The effect of 17-phenyl-$PGF_{2\alpha}$ ...rally resembled that of $PGF_{2\alpha}$ although a "two peak effect" was occasionally seen when ...rge doses of 17-phenyl-$PGF_{2\alpha}$ were given.

...$PGF_{2\alpha}$ and its analogues produced an initial fall in systemic arterial blood pressure followed ...long-lasting, dose-related and more marked increase (Fig. 6). The decrease in blood ...essure was often accompanied by bradycardia, whereas tachycardia was consistently seen ...ncomitantly to the rise in blood pressure.

In 2 guinea pig expts., $PGF_{2\alpha}$ and its analogues were administered also as aerosols. 16,16-...methyl-$PGF_{2\alpha}$ was again the most potent compound in eliciting an increase in insuffla...on pressure. Notably by this route of administration $PGF_{2\alpha}$ was as active as 15(S)15-...ethyl-$PGF_{2\alpha}$ and 15(S)15-methyl-$PGF_{2\alpha}$ methyl ester and it was considerably more ...tent than 17-phenyl-$PGF_{2\alpha}$. No effect or a slight decrease in systemic arterial blood pres...re was observed on aerosol administration. The heart rate was not influenced.

Discussion

...ecent observations on uterine and gastro-intestinal smooth muscle (Wiqvist *et al.* 1973, ...ström *et al.* 1973, Robert 1976, Nylander and Andersson 1976, Karim and Fung 1976) ...ave shown that methylation of primary prostaglandins at carbon 15 or 16 markedly in-

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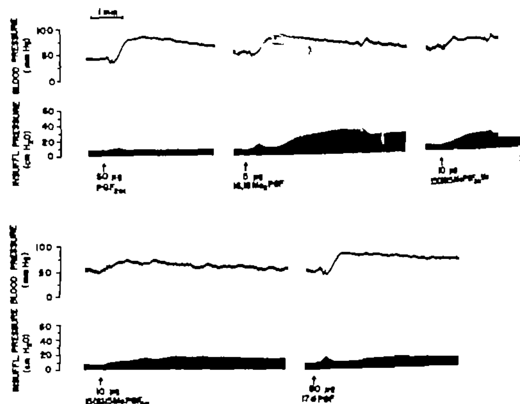


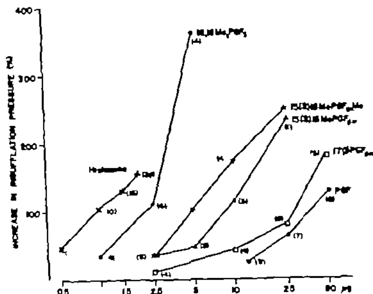
Fig. 6. Effects of i administration of $PGF_{2\alpha}$, 16,16-dimethyl-$PGF_{2\alpha}$, 15(S)15-methyl-$PGF_{2\alpha}$ methyl
15(S)15-methyl-$PGF_{2\alpha}$ and 17-phenyl-$PGF_{2\alpha}$ on the systemic blood pressure and tracheobronchial
tion pressure i an anaesthetized guinea pig

creases their potency and/or duration of action. Our data extend these findings to the gu
pig airway tree. Irrespectively of route of administration i v injection or aerosol admi
tion 16,16-dimethyl $PGF_{2\alpha}$ proved a very potent bronchoconstrictor being many t
more active than $PGF_{2\alpha}$. 15(S)15-methyl $PGF_{2\alpha}$ was also considerably more potent tha
parent compound. This was found to be the case both for the free acid and for the m
ester indicating that the C terminal is not essential for the bronchoactivity of $PGF_{2\alpha}$

Even more striking than the potency was the long duration of action of 15(S)15-met
$PGF_{2\alpha}$ and 16,16-dimethyl $PGF_{2\alpha}$. While the effect of i v administrated $PGF_{2\alpha}$ wa
terminated within 1 min these two analogues produced a sustained increase in insuffl
pressure of at least 15 min duration

Since the lungs contain large amounts of PGDH (Änggård *et al* 1971) and since pr
prostaglandins are inactivated to more than 90 per cent by one single passage through
lung circulation (Ferreira and Vane 1967) the increased potency and duration of actio
the analogues most likely reflects protection against PGDH (*cf* Weeks *et al* 1973 Sun
1976) In this context it is worth noticing that asthmatic patients are markedly hyper
tive to the bronchoconstrictor action of $PGF_{2\alpha}$ (Mathé *et al* 1973) The clinical use o
analogues, in particular 15(S)15-methyl-$PGF_{2\alpha}$ and 16 16-dimethyl $PGF_{2\alpha}$ *e.g.* as ab
ficants, involves a potential risk in such patients.

The bronchodilator activity of the PGEs and their analogues in guinea pig was e



; 7 Dose-response relations for the action of administered histamine, 16,16-dimethyl-PGF$_{2\alpha}$, 15(S)15-thyl-PGF$_{2\alpha}$ methyl ester 15(S)15-methyl-PGF$_{2\alpha}$, 17-phenyl-PGF$_{2\alpha}$ and PGF$_{2\alpha}$ on the tracheobronchial inflation pressure in the guinea pig. Mean values are presented. The number of expts are given within ackets

ed from their capacity to inhibit the bronchoconstrictor action of i.v. administered histamine. In confirmation of previous reports (Main 1964 Large *et al.* 1969 Rosenthale *et al.* 1970), PGE$_1$ and PGE$_2$ were potent bronchodilators, both when given i.v. and as aerosols. one of the PGE-analogues were significantly more active than the parent compounds. In ct, tw of them, 15(R)15-methyl-PGE and 17-phenyl-PGE$_2$, were less active than PGE$_2$. his indicates that the position of the 15 hydroxyl is essential for the bronchoactivity of GE$_2$ (*cf.* Rosenthale *et al.* 1976) and that a phenyl group at carbon 17 decreases the activity f PGE$_2$, possibly in conjunction with poor protection against PGDH.

On the other hand, with the exception of 17-phenyl-PGE$_2$, the PGE-analogues had a onger duration of action than the parent compounds, indicating that they were at least artially protected against PGDH. Their true duration of action was not established, however. Such an evaluation appears highly hazardous since PGEs have been shown both to nhibit and to enhance the effect of bronchoconstrictor agents (Main 1964). I other words, the response to a bronchoconstrictor substance recorded after PGE administration is the et result of two opposing factors.

Unexpectedly some of the PGE-analogues, notably 16,16-dimethyl-PGE and 16,16-imethyl-PGE$_2$, produced bronchoconstriction when tested on isolated human bronchi. This as not due to a low preset of bronchial tone, since the tone had been adjusted to approximately 50 per cent of the contraction–relaxation span. Nor could the effect be xplained in terms of decreased capacity to raise the cyclic AMP levels, since subsequent administration of isoprenaline promptly relaxed the preparation.

PGE and PGE were consistently relaxant although in approximately half of the ex the relaxation to PGE_1 was followed by constriction In fact, it has been reported that K sometimes may cause bronchoconstriction also *in vivo* in man (Mathé *et al.* 1973, Sm 1974 Mathé and Hedqvist 1975). The generation in lung tissue of both PGF and P compounds capable of producing bronchoconstriction is therefore worth consider when the role of PGs as possible mediator substances in obstructive lung diseases is discu

Certainly increased potency and prolonged action are two highly desirable attribu PGEs intended for therapeutic use as bronchodilators. Yet, it is questionable if even t improvements of the native PGEs warrant their clinical introduction. During recent ye number of non-irritating, relatively selective β_2-adrenoceptor stimulating agents have b introduced On aerosol administration these compounds have a sustained potent bron dilator action in concentrations that have little side-effects. Moreover tachyphylaxis increased tolerance does not seem to develop to any significant extent when they are us bronchodilators in asthmatic patients (Svedmyr *et al* 1974). Therefore, the PGEs or t analogues tested in the present study do not seem to possess any outstanding featu these respects. In fact, the irritancy and the potential bronchoconstrictor properties of compounds may even imply a risk in clinical practice.

This study was supported by grants from Swedish National Association against Heart and Chest Di and from Magnus Bergvalls Stiftelse.

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Acta physiol. scand 1977 100 182-186

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Brain Energy Metabolism in Angiotensin-Induced Acute Hypertension in Rats

By

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Received 28 October 1976

Abstract

JOHANSSON B B and B K. SIESJÖ. *Brain energy metabolism in angiotensin-induced acute hypertension in rats* Acta physiol. scand 1977 *100* 182-186.

In order t study if rapid elevation of blood pressure is associated with cerebral ischemia, ane[illegible] (70 % N O) and artificially ventilated rats were subjected t angiotensin-induced hypertension. After [illegible] hypertensive period, cerebral cortex tissue was frozen *in situ* for subsequent measurements of labile [illegible] lytic metabolites, ammonia, and organic phosphates. The degree of hypertension induced, [illegible] evidence of blood-brain barrier damage in 7 of 8 rats, did not affect the tissue concentrations of [illegible] metabol tes. It is concluded that ischemia does not contribute to the barrier damage, nor is it likely [illegible] the cause of the clinical symptoms that may occur in conscious rats i the same experimental model

Ke ords Angiotensin, blood-brain barrier energy metabolism, hypertension, ischemia

Acute hypertension induced by various vasoactive substances or by compression of t[illegible] thoracic aorta results in multifocal protein extravasation in the brain, *i.e.* a breakdown [illegible] the blood-brain barrier (BBB) to protein, presumably caused by distension of the ves[illegible] (Häggendal and Johansson 1972) The hypotheses of vasospasm and ischemia as the ca[illegible] of the permeability disturbances seem unlikely for the following reasons

1 Extravasation of protein tracers are seen in animals killed within 30 s after the pres[illegible] increase Ischemia of such a short duration does not result in any increased permeabi[illegible] (for discussion see 1974 a)

2. In the acute stage of hypertension areas with BBB dysfunction have higher regi[illegible] blood flow than nearby regions in studies using H-ethanol (Johansson 1974 b) or label[illegible] microspheres (Bill and Linder 1976) as blood flow indicators.

3 Vasodilatation increases the vascular damage (Johansson 1974 c, 1976)

Most studies of cerebral circulation in acute hypertension show an increase of the to[illegible] cerebral blood flow (Ekström-Jodal *et al* 1971/72, Strandgaard *et al.* 1973 1976). How[illegible] the blood flow may be heterogenous and some experimental studies of regional cereb[illegible] blood flow have indicated areas with markedly decreased flow [illegible] ins *et al.* 19[illegible]

ɛ1 nsdale *et al.* 1974)· In fact, the latter authors postulate—but have not shown—that those the areas with increased permeability

When conscious rats are subjected to acute angiotensin-induced hypertension some animals velop seizures within a few minutes of the pressure increase (Johansson and Henning 1976). e brains from such animals have regions with Evans blue-albumin extravasation. However BB dysfunction does not necessarily lead to clinical symptoms and we still do not know at initiates the symptoms. One possibility is focal edema, but clinical signs may occur ore a marked edema is present. For reasons mentioned above, it seems unlikely that hemia *per se* is the cause of the permeability disturbance. On the other hand it is possible it ischemia could be of importance for the appearance of neurological symptoms.

To shed light on the controversy whether or not ischemia occurs in the initial stage of ute hypertension biochemical studies of tissue metabolism are needed. The methods to idy regional cerebral blood flow available t present do not allow a resolution at the pillary level, and minute areas of low blood flow may be hidden in high-flow" areas. e objective of the present study was to examine if any biochemical signs of ischemia are ent in the brain in acute hypertension. According to Dinsdale *et al.* (1974) low flow areas most numerous 5 min after attaining the maximal systolic blood pressure. We therefore ded to study the concentration of labile cerebral metabolites, reflecting the energy state he tissue, in animals killed 5 min after an acute blood pressure increase.

Material and Methods

e Wistar rats (230–300 g), 8 experimental animals and 4 controls, were initially anesthetized with 2.5- halothane. When asleep, the animals were tracheotomized and immobilized with tubocurarine chloride maintained artificially ventilated on 70 N_2O and 30 O_2. Both femoral arteries were cannulated blood pressure recording via pressure transducer and for anaerobic blood sampling. One femoral n was cannulated for injections of drugs. The temperature as measured in the rectum. A longitudinal sion was made over the skull bone to accomodate plastic funnel for later freezing of the brain *in situ* rlin *et al.* 1973)

Rats anesthetized with nitrous oxide have higher mean arterial blood pressure (MABP) than conscious barbiturate-anesthetized rats and the initial MABP was 143±2 mmHg (M±S.E.) As we know from lier studies that it not only the blood pressure level but also the magnitude of the pressure increase that important for the appearance of BBB dysfunction in acute hypertension (Häggendal and Johansson 72), the blood pressure was first lowered to 92±5 mmHg in the experimental group and to 91±1 mmHg the control group by administration of dihydralazine. 20 min later the blood pressure was increased an injection of 5 μg angiotensin

Following freezing of the brain *in situ*, the tissue was chiselled out at liquid nitrogen temperature and red at 80 C until analyzed For analysis, cerebral cortex tissue (fronto-parietal regions) was separated 22°C and extracted with HCl-methanol The subsequent treatment of the extracts, and the enzymatic, orometric techniques (Lowry and Passonneau 1972), were as described previously (Folbergrová *et al.* 72 and b) The following metabolites were analyzed, glycogen, glucose-6-phosphate (G-6-P), pyruvate, ctate, ammonia, phosphocreatine (PCr), creatine (Cr), ATP ADP and AMP

The BBB as not studied in brains used for metabolic studies because the presence of Evans blue may terfere with the results of the analyses. Moreover areas of extravasation are multiple but small in the rat ain, and, because of high tracer concentration in the blood, not easy to detect from the surrounding sue in brain that has not been perfused. Previous studies have shown that areas of increased permeability evelop in animals treated in comparable way In order to exclude the possibility of differences due to at strain or to experimental procedures, comparable blood pressure increase was induced in 8 additional animals, treated as described above but given Evans blue as BBB tracer The brains were perfused *in situ* 5 min after the increase in pressure

TABLE I Body temperature and mean arterial blood pressure, as well as arterial P_{O_2}, P_{CO_2} and pH control and (5 min) hypertensi groups. Also given are the maximal pressures obtained in the latter group The values are means ± S E.

Group	Temp. °C	MABP mmHg	MABP (max.)	P_{O_2} mmHg	P_{CO_2} mmHg	pH
Control (n = 4)	36.8 ±0.1	91 ±12		115 ±7	35 5 ±0 6	7.3[illegible] ±[illegible]
Hypertension (= 8)	36.8 ±0.1	92 ±5	163 ±3	1[illegible]2 ±2	36.7 ±1 1	7.3[illegible] ±[illegible]

Results

The physiological variables in control and hypertensive rats are shown in Table I. Bo[illegible] temperature, as well as arterial PO_2, PCO and pH were similar in the 2 groups. T[illegible] increase in mean arterial blood pressure, following *i.c* injection of angiotensin was 7[illegible] 3 mmHg.

Table II contains data for the labile cerebral metabolites. As can be seen, induced hyp[illegible] tension did not alter the tissue concentrations of glycolytic metabolites, of ammonia, or [illegible] labile organic phosphates

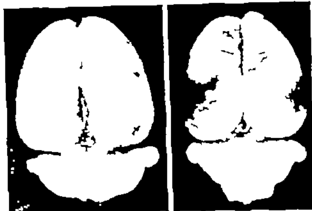
7 of the 8 brains that were used for BBB studies showed areas of protein extravasati[illegible] Fig. 1 illustrates the pattern of Evans blue-albumin leakage seen in 5 of the brains. In [illegible] brains the extravasation was more extensive as shown in Fig. 2.

Discussion

It is well known that complete cerebral ischemia, or a reduction of cerebral blood flow below about 50% of control, is accompanied by marked perturbations in a number of lab[illegible] cerebral metabolites (for references, see Nilsson *et al* 1975) The earliest and largest chang[illegible]

TABLE II Labile metabolites in cerebral cortex f control and hypertensive rats. The values are mean[illegible] S E. in μmol g^{-1}

	Control (= 4)	Hypertensi[illegible] (n = 8)
Glycogen	2.18±0.18	[illegible]51±0.13
Glucose	5.26±0 38	6.05±0 14
G-6-P	0.094±0 002	0 091±0 003
Pyruvate	0.134±0 010	0.1[illegible]8±0.006
Lactate	1 72±0.20	1 64±0.12
Lactate/Pyruvat	12.7±0.5	12 7±0.3
NH^+	0.23±0 01	0.25±0 01
PCr	4.45±0 05	4 40±0.09
Cr	5 77±0.17	5 73±0 08
ATP	[illegible]94±0.01	2.99±0.01
ADP	0.269±0.010	0 27[illegible]±0.006
AMP	0.038±0.002	0 037±0.001



1 and 2 Rat brains with areas of Evans blue-protein extravasation (dark spots) in cortex after amphetamine-induced acute hypertension (5 min)

Fig. 1 Fig. 2

... in the tissue concentrations of PCr lactate, and AMP as well as in the lactate/pyruvate ratio while more pronounced degrees of ischemia also cause depletion of ATP glycogen, glucose and G-6-P and accumulation of ammonia. It is of special interest that, during severe ischemia, the lactate concentration may increase 10-fold, and the AMP concentration 50-fold. Thus, even if only a few percent of the tissue is ischemic, analyses on whole tissue would reveal increased concentrations of these metabolites.

The present expts. have demonstrated that 5 min of hypertension of a degree that leads to extravasation of Evans blue does not measurably affect the tissue concentrations of labile energy metabolites that are sensitive indicators of brain ischemia. This makes it unlikely that the neurological symptoms that may occur in conscious rats within the first few minutes of an abrupt blood pressure increase (Johansson and Henning 1976) are provoked by ischemic metabolic changes. Furthermore, the results seem to exclude the possibility that the extravasation of protein during hypertension is caused by a severe reduction in blood flow (see Introduction) and rather corroborate the assumption that the extravasation is initiated by vascular distension induced by the rapid pressure increase. Further evidence that increased permeability can be mechanically induced by vasodistension has been obtained in other animal models of BBB dysfunction. Thus, in bicuculline-induced seizures and in severe hypercapnia, two conditions with extensively dilated cerebral vessels, the protein extravasation is correlated to the blood pressure level and can be prevented by maintaining a low blood pressure (Johansson and Nilsson, in press).

Excellent technical assistance was given by Gunilla Gidö. This study was supported by grants from the Swedish Medical Research Council (Project No. 14X 263), the Swedish National Association against Heart and Chest Diseases, and from U.S. PHS No. 2 R01 NS07838-07 from N.I.H.

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Muscle Spindle Activity in Man during Standing

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Received 17 November 1976

Abstract

BURKE, D. and G. EKLUND. *Muscle spindle activity in man during standing.* Acta physiol. scand. 1977. *100*. 187–199.

In standing human subjects, muscle spindle activity was recorded from peroneal nerve fascicles innervating anterior compartment muscles. Provided that the receptor-bearing muscle remained relaxed, the basal activity and responses to stretch seen in multi-unit and single unit afferent recordings were stable and not altered by eye-closure or assistance to balance. The discharge frequencies of single afferent fibres were similar to those recorded at comparable muscle lengths in relaxed reclining subjects. By analogy to findings in reclining subjects, these results suggest that, during standing, the background fusimotor drive to relaxed muscles is negligible. Backward body sway can induce a sway-stabilizing reflex contraction in the muscles of the anterior compartment. Such reflex contractions were accompanied by increased muscle spindle activity, the intensity of which appeared to be related to the intensity of the skeletomotor contraction. When balance was assisted by holding a support, swaying movements of similar or greater amplitude and velocity did not produce reflex activity and the spindle response was of low frequency. It is concluded that the sway-stabilizing reflex contraction operates in alpha-gamma linkage, and that these contractions are not generated by segmental stretch reflex pathways.

Studies of muscle spindle afferent activity in sitting or reclining human subjects have demonstrated that there is normally a tight functional linkage between skeletomotor and fusimotor activity. Thus, isometric voluntary contraction of a muscle is accompanied by acceleration of spindle discharge consistent with activation of the fusimotor system (Hagbarth and Vallbo 1968, Vallbo 1971, 1974 b, Hagbarth, Wallin and Löfstedt 1975), and, conversely in relaxed muscles there appears to be no significant background fusimotor drive (Hagbarth, Hongell and Wallin 1970, Vallbo 1970, 1974 a, Wallin, Hongell and Hagbarth 1973), Hagbarth, Wallin, Burke and Löfstedt 1975, Burke *et al.* 1976 b). Thus far the only documented exceptions to this general principle are the tendon jerk (Szumski *et al.* 1974, Hagbarth, Wallin, Lofstedt and Aquilonius 1975) and the form of tonic stretch reflex induced by muscle vibration (Burke *et al.* 1976 b). It seems reasonable that the spindle afferent activity responsible for the stretch reflex should not excite gamma motoneurones since otherwise the spindle activity might be self-perpetuating.

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muscle, attempts were made to ensure that the contractions were, as far as possible, isometric. This ...ace required the experimenters to restrain the subject' foot and toes.

...ments of the ankle joint were monitored using goniometer the arms of hich were secured to ...t and leg. The electromyogram (EMG) of the muscle innervated by the penetrated fascicle (here... "the innervated muscle") was recorded routinely using either surface electrodes or pair of tungsten ...s insulated to ithin 3–5 mm of the tips. On some occasions, the EMG of triceps surae was similarly ...ed. Because of the prominence of sympathetic activity in muscle nerve fascicles of standing man ...(results), the electrocardiogram (ECG) was also recorded in 12 expts. Data were monitored on an ...scope during the experiment and stored for subsequent analysis on Precision Instruments PI-... or Sangamo Sabre VI tape-recorder. The analysis of neural activity was performed using procedures ...ially similar to those described previously (Hagbarth and Vallbo 1968 Hagbarth, Wallin and Löfstedt ... Burke *et al.* 1976).

...asic assumption underlying the use of multi-unit recordings is that the neural activity is dominated ...uscle spindle afferents. This assumption has been substantiated in the studies of Hagbarth, Hongell ...Wallin (1970) and Wallin, Hongell and Hagbarth (1973). In addition, in the study of Vallbo (1974), ...verage position sensitivity of pooled sample of single spindle afferent fibres was found to correspond ...ly to that estimated from multi-unit recordings. In 3 of the present series of expts electrically induced ...le twitches confirmed that the respective multi-unit recordings were dominated by muscle spindle ...ent activity.

Results

...ti-unit afferent activity

...nding that the subject remained reasonably steady and the muscle innervated by the ...etrated fascicle remained relaxed, the level of integrated neural activity during quiet ...ding was stable and not subject to spontaneous fluctuations part from those arising ...n the activity of postganglionic muscle nerve sympathetic fibres. Fluctuations could ...etimes be produced by eye-closure or changes in trunk and upper limb posture, but ...e changes were always associated with mechanical events as recorded by the standing ...tform or the goniometer at the ankle, and were occasionally associated with a brief burst ...EMG activity in the muscles of the anterior compartment.

The sympathetic activity could be identified by the spontaneously occurring brief rhythmic ...rsts of neural activity synchronized to the pulse. Such activity waxed and waned inter...ttently but tended to increase during long expts. with increasing discomfort and f tigue. ...e features of this activity were typical of those previously documented for sympathetic ...erent activity in muscle nerve fascicles (Delius *et al* 1972). The intensity of sympathetic ...tivity was such that it sometimes proved difficult to find intrafascicular sites from which ...oprioceptive afferent activity could be led off uncontaminated by the rhythmical bursts. ...ch problems had not been encountered in the four subjects who had served in earlier ...udies of proprioceptive afferents in a reclining position (Hagbarth, Wallin, Burk and ...östedt 1975 Burke *et al* 1976 a, b), suggesting that the intensity of sympathetic outflow ...as accentuated in the upright posture. In Fig. 1 C and 4 C D the level of neural activity ...disturbed intermittently by sympathetic bursts

The responses to muscle stretch produced by either passive movements of the ankle joint ...r by maintained pressure on the tendon of the appropriate muscle were dominated by ...ynamic activity. Tendon taps delivered manually produced prominent bursts of neural ...ctivity (Fig 1 A) Slow movements of the ankle joint usually produced little neural activity provided that the innervated muscle did not contract), and faster movements generated ...ctivity hich subsided on completion of the stretching phase. Ramp stretch of the innervated ...

The act of standing creates different demands on the neural machinery and it would imprudent to assume that identical mechanisms operate in standing man as in the subject The stretch reflex has been considered a mechanism fundamental to the main of the upright posture (Roberts 1967) but a powerful antigravity mechanism does not sarily confer stability (Purdon Martin 1967) Indeed, in standing man, weight-bearing largely dependent on passive mechanical factors (Hellebrandt, Brogdon and Tepper Kelton and Wright 1949) although active muscular forces are required to maintain fixation, particularly at the ankle joint where forward rotation is opposed by contract the calf muscles (Smith 1957) Backward sway is counteracted by an involuntary contra of the muscles of the anterior compartment of the leg, which muscles, unlike those of calf are electrically silent in the average standing position, but are brought into when the vertical projection of the centre of gravity passes behind the axis of the joints.

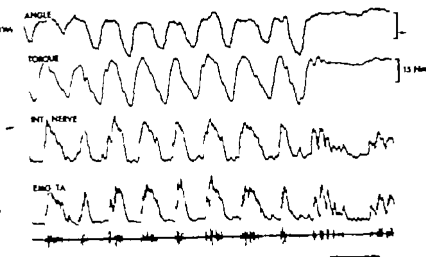
The present study was undertaken to investigate the role of the fusimotor innervation the muscles of the anterior compartment in the maintenance of postural equilibri standing man, with particular reference to whether there is evidence of background motor drive to relaxed muscles, and whether when the muscles contract to help equilibrium, spindle endings are activated (as in a voluntary contraction) or unloaded in a segmental stretch reflex)

Methods

Data were obtained from 15 expts. in 6 normal subjects aged 20–39 years, all of whom gave consent to the experimental procedure. During relaxed standi g muscle afferent activity was recorded the peroneal nerve at the fibula head usi g tungsten micro-electrodes inserted percutaneously into innervating the muscles of the anterior compartment of the leg. The techniques used have been in detail elsewhere (Hagbarth and V llbo 1968 Vallbo 1970, 1971 1974 a, Hagbarth, Wallin and 1975). Recordings were made from muscle nerve fascicles innervating tibialis anterior (TA) on 12 occ extensor hallucis longus (EHL) and extensor digitorum longus (EDL) on 9 occasions, and extensor brevis (EDB) n one occasion Multi-unit afferent activity was recorded from most fascicles, and, tion, single unit recordings were obtained from 56 afferent fibres innervati g muscle mechanorec Due to the normal instability of stand g subjects, only limited data could be obtained from each of units, and the identification procedures were incomplete. Some units were subjected to an electrical duced muscle twitch contraction, and could therefore be identified as of spindle or tendon organ but for the majority of the afferent units definitive classification was not possible.

The subjects were requested t mai tain relaxed stance with feet orientated comfortably and metrically on force platform designed to measure the antero-posterior component of the supporting (Eklund and Löfstedt 1970 Eklund 1972). Since prolonged standing in the same position is (Smith 1953) and proved to be particularly rdoom, experiments were interrupted periodically, and subjects allowed to sit. Additionally when the subject was upright, a rope hanging from the ceiling used as support during the sometimes lengthy searching fo suitable intrafascicula recording sites. rope was also used when testing the effects of an external assistance t balance on neural activity

The afferent response t tendo percussion was assessed by tapping the appropriate tendon, with a fi ger Muscle stretch was produced either by active and passive sw yi g movements of the resulting in rotation of the ankle joi t, or by maintained pressure pplied bruptly to the tendon appropriate muscle. With the former method the amplitude nd rate of stretch could be measured nless the subject balance was supported (by the rope) swaying movements initiated n involuntary correction (TA activity) once the centre of gravity had passed fa enough backw rds. With the latter although the parameters of the stretchi g stimulus could not be quant tated, the stimulus delivered muscle approximated ramp stretch more closely thus allowing easier differentiation of the dynamic static components of the afferent response. When testing the effects of voluntary contractions of the



Responses to body sway. Backward sway is indicated by an upward deflection of the angle and records. The neural response is more closely related to the reflex EMG activity than to the amplitude of stretch. The integrator time constants (nerve and EMG) are 0.1 . As in subsequent figures th... arrow on the angle calibration scale indicates the average standing position of the ankle joint.

the relaxed standing position, the muscles of the anterior compartment are usually ...ically silent, but with swaying movements EMG activity appears once the vertical ...ction of the centre of gravity has passed behind the transverse axis of the ankle joints. ...rallel with the EMG activity backward sway generated intense afferent neural activity ...nset of which either accompanied or lagged slightly behind the first EMG potentials ... 2). These responses to sway were involuntary and incoercible. The intensity of the ...al discharge was correlated to the intensity of the reflex EMG activity recorded from ...appropriate muscle. Similar responses were recorded whether the swaying movement ...initiated actively by the subject or passively by experimenters. However if swaying ...ements were performed while the subject was supporting balance by grasping the rope, ...ements of greater amplitude and velocity could be performed without provoking reflex ...G activity. In this case the afferent neural activity was of lower intensity and more ...rly dependent on the amplitude and velocity of the swaying movement (*cf.* Fig. 7 B).

...gle unit recordings

...cordings were obtained from 56 single afferent fibres innervating muscle mechanorecep... endings, the identification of which is incomplete (see Methods). However by analogy ...h previous studies in reclining subjects, it is probable that by far the majority of such ...chanoreceptive afferents were from muscle spindle endings, most commonly the primary ...ding (*cf.* Vallbo 1970). Certainly it is inconceivable that the single unit population did ...t contain a good sampling of spindle afferent activity. For 17 units a spindle origin was ...tablished by either (I) the typical pause in discharge during an electrically induced twitch ...ntraction with discharge during the relaxation phase of the contraction (11 units), or (II) a

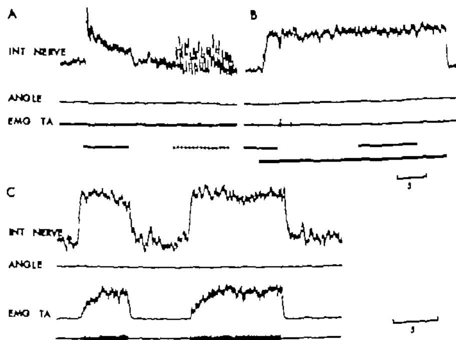


Fig 1 Multi-unit neural responses from TA. A. Responses to maintained pressure on TA tendon indicated by the solid bar and to repeated tendon percussion as indicated by the dotted line. B Res to vibration of ampl tude 1 5 mm and frequency 100 H applied t TA tendon during the lower thick Duri g the periods indicated by the thinner bars the subject assisted balance by holding a rope C Res t isometric voluntary co tractions as indicated by EMG activity A, B and C are from different ments. In each record the i tegrato time constant was 0 1 s, and the subject was stand g erect at a position. Irregularities in the integrated neural activity (particularly in C) are mostly due to EMG bursts.

muscle produced by pressure on the appropriate tendon evoked a prominent dy burst of activity as stretch was applied a rapid decay of activity during maintained st and occasionally a transient decrease in activity below the basal level on release. In cicles there was a significant level of maintained activity in response to static stretch, b all the integrated level was less than half that produced by the dynamic phase of s (Fig. 1 A)

The level of neural activity recorded when the subject was standing freely with no ance to balance did not differ from that recorded when the subject s balance was as by holding a support. Grasping the support minimized the mechanical irregulariti corded by the standing platform, while releasing the support sometimes produced tra postural disturbances and occasionally the subject assumed a new mean posture. from changes induced by these mechanical events, the basal level of neural activity an responses to ramp stretch were identical whether standing with or without assistan balance. Similarly vibration at frequencies 100–200 Hz and amplitude 1.5 mm appl the tendon of the innervated muscle evoked an intense sustained afferent barrage whic the same whether the subject was standing with or without assistance to balance (Fig. Apart from muscle vibration, the only other way of inducing a well maintained n discharge was contraction of the innervated muscle, be the contraction voluntary (Fig. or induced reflexly by swaying movements (Fig. 2)

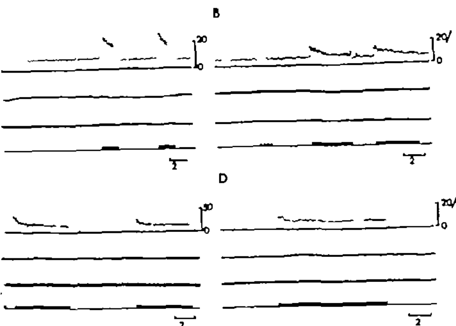


Fig. 4. Responses of single units to maintained ramp stretch, illustrating predominantly dynamic response units with and without background discharge (A and C), and predominantly static response for units with and without background discharge (B and D). In each record, the traces are, from above, instantaneous frequency plot; ankle joint angle EMG event marker. In B, 4 tendon taps were delivered before the ramp stretch. In each record the subject was standing freely in relaxed position. Sympathetic bursts disturb frequency plot in C and D.

…ency in the sway-induced reflex was similar to or only slightly greater than that recorded … swaying movements not associated with reflex EMG. However, since skeletomotor activity …ot accompanied by significant fusimotor drive tends to unload spindle endings and reduce their firing rate (Hagbarth *et al.* 1970, Vallbo 1974 b, Burke *et al.* 1976 b), the findings with these 3 units (assuming that all 3 were spindle endings) remain suggestive of fusimotor activation accompanying the skeletomotor contraction.

The presence of significant fusimotor drive accompanying the sway-induced reflex EMG activity is demonstrated in Fig. 6 and 7 for 2 identified spindle endings. In Fig. 6 A the subject was supporting his balance by *grasping a rope hanging from the ceiling, and, in the* absence of reflex EMG the discharge rate of the spindle ending was relatively low. In B, swaying movements of the same amplitude and velocity generated reflex EMG activity and greater spindle response, the intensity of which varied with the intensity of skeletomotor activity. In Fig. 7 reflex contractions induced by body sway were associated with intense activation of a spindle ending in TA. However when the subject supported himself using the rope, swaying movements of larger amplitude and velocity could be performed without reflex contraction, and the spindle response consisted of a relatively low frequency discharge.

In both reflex and voluntary contractions, the onset of skeletomotor activity preceded

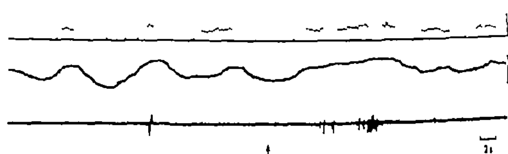


Fig. 3 The effect of assistance to balance on the discharge of a spindle ending in TA. The subject swaying slightly backwards and forwards, initially with balance assisted by grasping the rope, which was released at the vertical arrow. Apart from irregularities associated with EMG activity the frequency of the ending does not change. Traces are, from above, instantaneous frequency plot, ankle joint, EMG of TA.

burst of impulses on relaxation of an isometric voluntary contraction (6 units). Two were identified as of tendon organ origin on the basis of their discharge during the phase of an electrically induced twitch contraction.

Of the 56 fibres, 33 (including 11 of the 17 identified spindle afferents) had no basal discharge in the relaxed standing position, 10 had a basal discharge rate of 0–5 Hz but this was usually irregular and poorly sustained, and 12 had a basal rate of 5–10 Hz (cf. Fig. 4). One unit had a basal rate of 13–14 Hz, the only unit to exceed 10 Hz. The discharge rate of these fibres did not alter with eye-closure or if the subject grasped a support to assist balance (Fig. 3), except in those cases where a mechanical disturbance was recorded by the foot. Similarly when firing was maintained or initiated by ramp stretch produced by pressure on the appropriate tendon or by tendon vibration assistance to balance did not alter the discharge rate.

Of 34 endings tested with "ramp stretch" 9 responded in a predominantly static manner, there being little dynamic response to the application of tendon pressure (Fig. 4 B). Four of these 9 endings were not basally active during quiet relaxed standing. Maintained static stretch generated sustained discharge frequencies of 10 Hz or less for 27 of the endings, as did slow stretching movements of the ankle joint with an additional 6 endings. The static discharge frequencies of the remaining 7 units exceeded 10 Hz, the highest frequency being 24 Hz. For 25 endings the dynamic response to ramp stretch was prominent, generating frequencies of 10–60 Hz (Fig. 4 A, C). The parameters of stretch produced by pressure on the tendon were not quantitated, but the significance of the present findings lies in the low discharge rates of all endings in response to static or slow stretch, and, for most of the endings, the contrast between the discharge rates produced by dynamic and static stretch.

With 35 units (15 of which were identified spindle afferents) the responses during voluntary contractions (Fig. 5 A) or sway-induced reflex contractions (Fig. 5 B) were studied. Accompanying both forms of skeletomotor activity afferent units accelerated to frequencies of 10–40 Hz, frequencies which greatly exceeded the basal discharge rate and, with 3 exceptions, the frequencies generated by static stretch or slow swaying movements in the absence of reflex contraction. For these 3 units, one of which is illustrated in Fig. 3, the discharge

C

2

100ms

Fig. 7. Response of spindle ending in TA during swaying movements, when standing without assistance balance (A) and when supporting balance by grasping the rope (B). In A and B, traces are, from above, instantaneous frequency plot; ankle joint angle; integrated EMG of TA (time constant 0.2 s); EMG of TA. In C, 3 electrically induced twitch contractions are superimposed to demonstrate the relationship between the neural response (upper trace) and twitch torque as recorded by the standing platform (lower trace).

53) and with more prolonged standing motor unit discharges in triceps surae gradually come grouped into a rhythm resembling tremor (Mori 1973, 1975). It could be that the underlying neural mechanisms may have altered during prolonged standing, but it seems unlikely that this factor would invalidate the general conclusions that can be drawn from the present study.

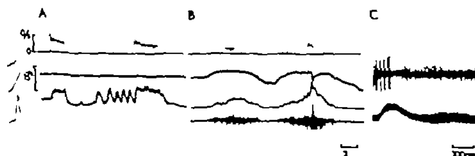


Fig. 8. The effect of contraction on the discharge of a tendon organ in EDL. A. Voluntary contraction. B. Sway-induced reflex contraction. In A and B, traces are, from above, instantaneous frequency plot; ankle joint angle, integrated EMG of EDL (time constant 0.2 s); EMG of EDL (in B only). In C, 3 electrically induced twitch contractions are superimposed to show the relationship between afferent response (upper trace) and twitch torque as recorded by the standing platform (lower trace).

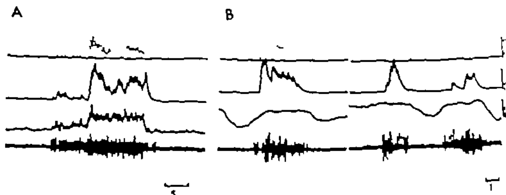


Fig. 5 The effect of contraction on the discharge of a spindle ending in TA. A. Voluntary contract The ending has no background discharge but is activated for the duration of the contraction B. s induced reflex co traction In A and B, traces are, from above, instantaneous frequency plot, s instantaneous frequency plot (time constant 0.5 s) Integrated EMG of TA (time constant 0.1 s) a kle joint a gle in B EMG of TA. In B, 5 s has been omitted at the break in the trace.

the acceleration of afferent discharge. The latency difference was variable, from neglig to 0.5–1 0 s, but was not studied systematically

The 2 identified tendon organs were relatively insensitive to passive stretch but were sensitive to muscle contraction, discharging in response to both voluntary contr (Fig. 8 A) and sway-induced reflex contraction (Fig. 8 B). Thus, the responses of identif spindle endings and tendon organs were qualitatively similar with both forms of ske motor activation.

Discussion

It must be conceded that the prolonged standing required to obtain suitable recordings does not represent a normal standing pattern and most subjects found the experim arduous. Man normally maintains the same stance for approximately half a minute (5–

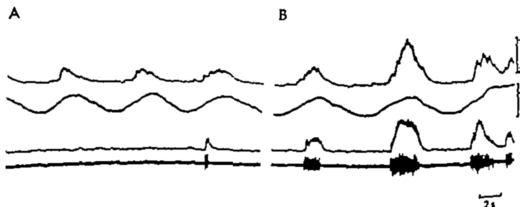


Fig. 6. Response of a spindle ending in TA during swaying movements performed when balance as s by holding the rope (A) nd after release of all support (B). Traces are from bo smoothed instant frequency plot (time constant 0.5 s) ankle joint angle Integrated EMG of TA (time constant 0.2), E of TA

refle contractions induced by body sway generated by the stretch reflex.

in tendon jerk reflexes and clonus (Szumski *et al.* 1974, Hagbarth, Wallin, Löfstedt ... Aniolonius 1975) and in the tonic vibration reflex (Burke *et al.* 1976 b), contractions ...ed by segmental stretch reflex mechanisms produce evidence of spindle unloading, ...ing that the phasic and tonic stretch reflexes operate predominantly on alpha rather ...mma motoneurones. Thus, the skeletomotor response reduces the afferent activity ...ated the necessity for the response (Burke *et al.* 1976 b). Since the reflex contractions ...d by body sway are organized in alpha-gamma linkage, these postural reflexes cannot ...lified as stretch reflexes of the type represented by the tendon jerk or the vibration-...d tonic stretch reflex. Instead, the findings are consistent with the view that the sway-...d contractions represent postural reflexes of a higher order, conveyed through supra-... centres.

...belief that the segmental stretch reflex is not the generator of the sway-induced con-...n is supported by the observations that the tendon jerk is suppressed (Elner *et al.* ... and the tonic vibration reflex is unobtainable (Eklund 1972, 1973) in the leg muscles ...ly standing man. Instead of inducing a localized autogenetic contraction, vibration of ...scles during unassisted standing produces wide-spread motor readjustments resulting ...ody sway which leads to shortening of the vibrated muscles (Eklund 1972, 1973). There ...e little doubt that these responses represent postural reactions of a higher order of ...lexity than segmental stretch reflexes, and they may well be mediated by the same ...segmental centres as are responsible for the sway-induced reflex contractions studied in ...resent paper

...e view that the falling reactions are generated mainly by vibration-induced activity in ...dary spindle endings (Eklund 1972, 1973) is consistent with the findings on the sen-...ty to vibration of human secondary endings (Burke *et al.* 1976). However the centres ...nsible for maintaining stable stance also receive and integrate afferent cues from other ...es, such as vestibular receptors (*cf* Nashner 1973), muscle spindle endings in many ...le groups (*cf* Eklund 1972, 1973) and the visual system (*cf* Romberg 1857). The modu-...n of a vestibular-induced response by the afferent activity evoked by ankle rotation ...hner 1973) demonstrates such an interaction for two of these sources of afferent in-...ation, while clinical experience with Romberg's test demonstrates the stabilizing po-...al of visual cues.

...cently Nashner (1976) demonstrated ... y-stabilizing response dependent on ankle ...tion in 5 out of 12 normal subjects, and suggested that the subjects used "long-latency ... ms) stretch reflexes to help reduce postural sway" However that the reflex output ...n equilibrium centres may result in contraction of muscle that happened to be stretched ...t not necessarily imply that reflex transmission occurred over stretch reflex pathways. ...thermore, since the reflex response was not confined to the muscle being stretched but ...ld be recorded simultaneously from other muscles, even, at times, the antagonist (Nash-... 1976 b), the phenomenon studied by Nashner cannot be considered stretch reflex as ... term is normally used. Nevertheless, if the responses described do indeed result from a ...ndle response to muscle stretch, Nashner's studies provide a further indication of the ...portance of muscle spindle activity to the centres governing postural equilibrium.

Fusimotor activity in standing man

In normal human subjects standing freely without support, muscle afferent activ reflected in multi-unit and single unit recordings from the peroneal nerve appear comparable with that recorded in reclining subjects at equivalent joint positions (H and Vallbo 1968 Vallbo 1970, 1974 a, Hagbarth, Wallin and Löfstedt 1973, 1976 a) Multi-unit afferent recordings were dominated by dynamically responding af and the level of neural activity was stable unless external disturbances occurred muscle contracted. Approximately 75–80% single afferent fibres were silent or had and usually irregular basal activity and the frequencies generated by maintained stretch were with one exception below 20 Hz. Eye-closure and external assistance to did not alter the afferent responses significantly The level of fusimotor drive is co to be very low or absent in relaxed reclining subjects (Hagbarth *et al.* 1970, Valb 1974 a, Wallin *et al* 1973 Hagbarth, Wallin, Burke and Löfstedt 1975), and, more r de-efferentation of spindle endings produced by complete block of the muscle ner local anaesthetic was found to have no effect on the basal firing rate or the response to (Burke *et al* 1976 b) From the present experiments, it seems reasonable to conclude standing man the background level of fusimotor drive to non-contracting muscles ligible.

In both multi-unit and single unit recordings increased spindle afferent activity panied the appearance of EMG potentials in the appropriate muscle whether the co contraction was initiated voluntarily or reflexly by backward body sway In both the increase in the afferent discharge occurred after the onset of EMG activity in ceptor bearing muscle. With maintained contractions, the intensity of the spindle r varied with the intensity of the EMG activity (*cf* Fig. 6 and 7) Such findings are dif explain by any mechanism other than activation of the fusimotor system along skeletomotor system, the intensity of the fusimotor drive being more than suffi compensate for the unloading effect of the skeletomotor contraction on the spindle These results resemble those seen when reclining subjects perform isometric vo contractions (Hagbarth and Vallbo 1968 Vallbo 1971 1974 b, Hagbarth, Wallin a stedt 1975) and it therefore seems reasonable to conclude that the act of standing does not involve a dissociation of the tight functional linkage between skeletomo fusimotor activity

In previous studies of vibration-induced falling reactions in standing subjects it wa that the magnitude of the body sway induced by muscle vibration was relatively inde of the pre-existing state of contraction of the vibrated muscles (Eklund 1972, 1973). assumption that in each situation vibration caused one to-one driving of spindle and that the magnitude of the induced sway depended on the overall increase in discharge frequency relative to the basal firing level these observations led to the p that during standing there may be a fairly constant level of fusimotor tone in leg independent of the state of contraction However it has since been shown that alpha- linked voluntary contractions enhance not only the basal discharge rate but also sponsiveness to vibration of spindle endings in the contracting muscle (Burke *et al* thus invalidating the assumptions that led to the original postulate.

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Does the segmental stretch reflex provide assistance to the sway-induced reflex co

Even though the sway induced reflex contraction is not generated through st pathways, it is possible that the co-activated spindle activity might provide s support to the contraction through segmental stretch reflex pathways, much a postulated for the co-activated spindle activity in a voluntary contraction (G Hagbarth *et al* 1970 Matthews 1972). For such assistance to be powerful the stretch reflex pathways would probably have to be increased along with the activ skeletomotor and fusimotor neurones, since stretch reflex gain appears to be l standing man (*cf* Eklund 1972, 1973 Elner *et al.* 1976) Gain consideration assistance could still be significant, since the background spindle discharge imp co-activated fusimotor drive allows spindle endings to reflect dynamic fre external load more faithfully The resulting irregularities in spindle firing may apparent at monosynaptic latency as an alteration in motor unit discharge patter Burg and Erbel 1973 Vallbo 1973 and unpublished observations).

In this respect the experiments of Shambes (1969) may be relevant Norm surae is active during standing to check the tendency to forward rotation at the (*cf* Smith 1957) Bilateral blockade of the sciatic nerves with local anaesthe deficits in postural stability at a stage sufficient to abolish the tendon jerk and in sensation without altering tactile sensation (Shambes 1969). Although some may be entertained about the selectivity of such blocks, the findings are consis view that intact fusimotor innervation and the resultant spindle afferent disc role in the maintenance of stable stance. The co-activated spindle discharge c stabilizing stance through segmental reflex pathways, through higher reflex me both

This study was supported by the Swedish Medical Research Council (Project No. B76-1
The uthors are grateful to Professor K. E. Hagbarth for advice and help throughout the s

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olesterol is poorly soluble in water but it can be dispersed as particulate suspension. der to stabilize these suspensions different means have been tried (Portman and Sinis- 1957 Nilsson and Zilversmit 1972, Sodhi and Kudchodkar 1973 b, Subbiah and Kukis

When particulate cholesterol is given i.v. to rats, the radioactivity of plasma reaches ximum immediately whereafter there is a rapid decline and a minimum follows in 30 (Nilsson and Zilversmit 1972). More than half of the injected radioactivity is found in ver (Avigan 1959 Appelgren 1967 Nilsson and Zilversmit 1972). The results of Nilsson Zilversmit suggest that it is the reticuloendothelial cells of liver and spleen which are nsible for the removal of particulate cholesterol from the blood. Gradually radio- ity reappears in the circulation and second maximum in plasma develops around 8– (Appelgren 1967 Nilsson and Zilversmit 1972). At this stage cholesterol is no more culate but bound to plasma lipoproteins It was suggested by Nilsson and Zilversmit the reappearance of cholesterol into the circulation would be due to its release from the ocytic cells as unesterified cholesterol, which is then esterified intravascularly or at r sites.

the present investigation the removal of particulate cholesterol from the circulation of rat was studied by following the radioactivity of cholesterol in blood and in different ns, viz. liver spleen, lungs and kidneys.

Material and Methods

nals Wistar rats weighing 200–350 g were used Each experimental group consisted of animals of e sex at time The rats were fed standard laboratory diet (Hankkija, Finland) *ad libitum*. All expts. started at 8–10 a.m. daily after an overnight fast

adioactive compounds The radioactive compounds were purchased from the Radiochemical Centre, rsham, England. They were used without prior purification. Cholesterol 4-^{14}C and 7-alpha-^{3}H (N) specific activities of 55 6–61 0 mCi/mmol and 500 mCi/mmol, respectively They were both found to be ast 96 5 pure or purer when analysed by thin layer chromatography

Preparation and administration of particulate cholesterol. Radioactive cholesterol (without carrier, if stated otherwise) was dissolved in one volume of ethanol in test tube, 10 volumes of water was added l the contents were mixed with vortex mixer A faint opalescence was usually seen, visible particles re not found. Immediately after the preparation 0 25–1 0 ml-aliquots of the dispersions were injected into vein of rats

Sampling techniques *Blood samples* were collected under light ether anesthesia from the tip of the tail. sma was separated by centrifugation after dilution of an aliquot of blood with 0 15 M NaCl-solution. the determination of the radioactivity of whole blood, measured aliquot was hemolyzed with 9 mM CO_3 and counted directly (Vaken 1975). For the calculation of blood radioactivity blood volume was umed to be 7 ml 100 g b wt (Gregersen and Rawson 1959).

Organ samples Animals were killed by cardiac puncture under ether anesthesia and blood was collected o EDTA-tubes Plasma and red blood cells were separated by centrifugation and the cells were washed ice with about 5 ml of 0 15 M NaCl-solution. Liver spleen, lungs and kidneys were removed and weighed. Organs were kept at 18°C until analysed

Organs were homogenized in 20 volumes of water with Potter-Elvehjem homogenizer Homogenates, sma and red blood cells were extracted with 20 volumes of chloroform-methanol 2 1 (v v). The extract as filtered and purified according to Folch *et al* (1957)

Determination of free and esterified cholesterol Free and esterified cholesterol were separated by thin yer chromatography (silica gel G, 0 25 mm). The plates were developed in petroleum ether diethyl ether- tic acid 85 15 1 (v v). Spots were visualized by iodine vapour and scraped either directly into counting als or into test tubes, where the support was extracted 3 times with chloroform-methanol 2 1 (v v)

Cholesterol extracted into hexane after alkaline hydrolysis (Abell *et al* 1952) was determined with

Acta physiol. scand. 1977 100 200-209
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The Fate of Intravenously Administered Radioactive Cholesterol Dispersion in the Rat

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Received 14 December 1976

Abstract

VIIKARI J., H SAARNI O RUUSKANEN and T NIKKARI *The fate of intravenously administered cholesterol dispersion in the rat* Acta physiol. scand. 1977 100 200-209.

The present work was aimed at studying the f rst stages of disappearance of particulate cholesterol from circulation. The distribution of radioactivity in blood, liver spleen, lungs and kidneys was determined at different time intervals after i v injections of aqueous-ethanolic dispersions of radioactive cholesterol into rats. More than 95 % of the dose disappeared from the circulation in 10 min. From 71 to 93 % of the label was found in the liver 5 min–2 h after the injection According to autoradiography most of the particulate cholesterol was immediately taken up by the liver parenchymal cells. There were only few grains over Kupffer cells. 2 min to 6 h after the i jection the highest specific radioactivity was in the free cholesterol fraction of the liver The specific activity of liver esterified cholesterol was 54 % of that of the free cholesterol at 1 h. They both declined gradually and reached the same level in 1 days. There was gradual rise of specific activities of plasma free and esterified cholesterol starting 30 min after the injection. Both reached a maximum in 6–16 h when the curve of plasma esterified cholesterol intersected that of liver esterified cholesterol. The radioactivity reappearing i plasma was associated with lipoproteins and red cells. The cholesterol of the high density lipoproteins had the highest specific activity The results indicate that particulate holesterol is taken up chiefly by the parenchymal cells of the liver and is subsequently incorporated int the plasma lipoproteins, most rapidly int the high density lipoproteins. A large fraction of plasma esterified cholesterol originates in the liver

Key words: aut radiography cholesterol chylomicrons Kupffer cells lipoproteins, low density lipoproteins, high density

For studies of the kinetics of cholesterol in the body radioactive cholesterol is usually injected intravenously either as "physiological" lipoprotein bound cholesterol or in an "unphysiological" particulate form Lipoprotein-bound radioactive cholesterol can be prepared *in vivo* or *in vitro* (Avigan 1959). Following its injection plasma radioactivity reaches a maximum immediately whereafter there is a decay which can be divided into 2 or 3 components indicating an equilibration between 2 or 3 hypothetical cholesterol pools (Nestel et al. 1969 Goodman *et al* 1973)

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[illegible]lesterol is poorly soluble in water but it can be dispersed as particulate suspension. [illegible]er to stabilize these suspensions different means have been tried (Portman and Sinis[illegible] 957 Nilsson and Zilversmit 1972, Sodhi and Kudchodkar 1973 b, Subbiah and Kuksis [illegible] When particulate cholesterol is given i.v. to rats, the radioactivity of plasma reaches [illegible]imum immediately whereafter there is a rapid decline and a minimum follows in 30 [illegible]Nilsson and Zilversmit 1972). More than half of the injected radioactivity is found in [illegible]er (Avigan 1959 Appelgren 1967 Nilsson and Zilversmit 1972). The results of Nilsson [illegible]ilversmit suggest that it is the reticuloendothelial cells of liver and spleen which are [illegible]nsible for the removal of particulate cholesterol from the blood. Gradually radio[illegible]ty reappears in the circulation and a second maximum in plasma develops around 8– [illegible] (Appelgren 1967 Nilsson and Zilversmit 1972). At this stage cholesterol is no more [illegible]ulate but bound to plasma lipoproteins It was suggested by Nilsson and Zilversmit [illegible]he reappearance of cholesterol into the circulation would be due to its release from the [illegible]ocytic cells as unesterified cholesterol, which is then esterified intravascularly or at [illegible] sites.

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modification of the ferric chloride method of Badzio and Boczon (1966). In this modification they heated at 60 C for 6 min in order to obtain full colour development.

Separation and isolation of lipoproteins The lipoproteins were fractionated by density gradient centrifugation ccording to Viikari and Pelliniemi (1974). The lipoprotein fractions were dialyzed at 4°C for at least 48 h and lyophilized. The lipids were extracted with chloroform-methanol according to Folch *et al* (1957)

Measurement f radioactivity Radioactivity was measured with Packard Tri-Carb liquid scintillation spectrometer (model 3320) equipped with an external standard. The scintillation mixture for lipids contained 0.4 % PPO (2,5-diphenyloxazole) and 0.01 % POPOP (4-methyl-5-phenyl oxazolyl)benzene in toluene. Maximum counting efficiencies for H and ^{14}C-cholesterol were 42 % and 96 respectively

The radioactivity from blood or plasma was also measured directly in a two-phase solvent system with internal standardization (Viikari 1975). Maximum counting efficiencies for ^{3}H and ^{14}C-cholesterol 30 % and 89 %, respectively

Radioautography *Organ samples* were fixed in cobolt-formalin (Jones *et al.* 1959). Sections of thickness were cut in a cryostat allowed to dry and coated with an emulsion (Kodak NTB-3) according Rogers (1967).

Cell smears of liver were prepared mainly according to Anderson (1953). A piece of liver was gently with forceps in saline. Unsuspended pieces were allowed t sediment and the canted and enriched (10 min, 100 g). The sediment was resuspended in a small volume of saline. the suspension was used for the preparation of the smear The smear was allowed to dry and with the emulsion.

The emulsion was allowed t dry about 5 h in a horizontal position. The glasses were week–5 months in − 20°C, developed, rinsed and stained with hematoxylin-eosin.

Results

Radioactivity of parti ulate cholesterol in blood and tissues

The radioactive particulate cholesterol disappeared rapidly from the blood (Fig. 1) a mean T of 1.3 min. More than 95 % of the radioactivity had left the circulation after the injection and there was a nadir after about 30 min, when only 1 2 % of

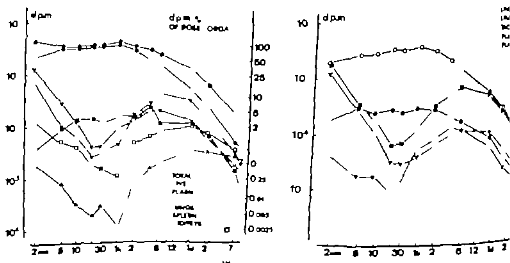


Fig. 1 Radioactivity of tissues after i. injection of particulate cholesterol. 20 μCi of H-cholesterol given to 13 rats. The organism was perfused with 0.15-M NaCl (50–100 ml) through the thoracic a remove the blood. Total = the sum of radioactivities in blood nd ll organs. FC = free cholesterol esterified cholesterol.



A series of radioautographic patterns of liver after … injection of particulate ^{3}H-cholesterol (20 μCi … was to each rat). Series a–d, frozen section technique, exposition time 2 weeks. Series e–h, cell smear … que, exposition time 5 months. a. 1.5 min, b. 10 min, c. 1 h, d. 24 h, … 1.5 min, f. 20 min, g. 2 h,

…ned in the circulation. Thereafter radioactive cholesterol re-entered the blood and a …mum was reached after 6–16 h followed by … slow decline.

…rtually all of the injected radioactivity was extractable from blood, liver, spleen, lungs … kidneys from 1.5 min to 2 h after the administration of the particulate cholesterol … 1 a). During … ts initial disappearance from the blood radioactivity accumulated in … and spleen. 5 min after the injection the liver contained approximately 71% of the …n. The radioactivity of the liver increased further during the first hour up to 93% of the … 4 per cent of the injected cholesterol was esterified in 1.5 min and 8–13% of the liver …oactive cholesterol was in esterified form from 1.5 min to 1 day after the injection (Fig. …). It was checked that no esterified cholesterol was present in the material injected. Al…ugh the maximum radioactivity taken up by the spleen was only 6 per cent of that of the …r, the shape of the radioactivity curve of the spleen resembled that of the liver and may …lect a partial similarity in handling of particulate cholesterol by these two organs.

About 1 h after the injection, coinciding with the start of the decrease of liver the radio…tivity in blood, lungs and kidneys (Fig. 1 a) began to rise again. The radioactivity found … lungs and kidneys may be due to blood remaining in these organs even after perfusion …th saline, whereas the majority of the radioactivity of plasma obviously originated in the …er.

…ioradiographic localization of particulate cholesterol in the liver

…ly few grains were seen over the Kupffer cells 1.5 min to 24 h after the injection of parti…late cholesterol (Fig. 2). On the other hand in liver parenchymal cells there was radio…ctivity already 1.5 min after the injection (Fig. 2). The labeling was at its maximum 10–60 …in after the injection, which is in good agreement with chemical data (Fig. 1). There was a

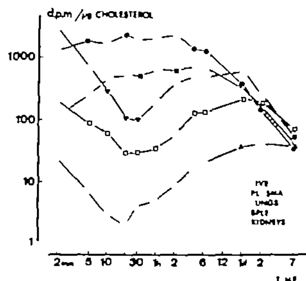


Fig. 3. Specific radioactivity of plasma and tissue cholesterol after i.v. injection of 3 … of particulate ³H-cholesterol.

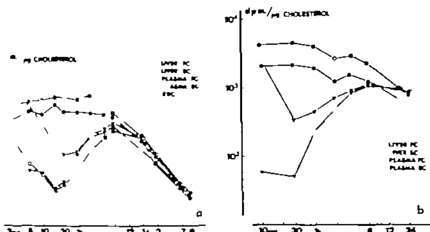
decrease in the number of grains at 6 h and only occasional grains were seen after … when the radioactivity of the liver had dropped to 14 % of the maximum value. The re… indicate that i.v. injected particulate cholesterol is taken up chiefly by liver parenchymal … rather than Kupffer cells.

Specific radioactivity of cholesterol reappearing into circulation

The specific activity of total cholesterol in the liver, plasma, spleen and lungs reache… equilibrium in 2 days after the injection of radioactive particulate cholesterol, wh… specific activity of the kidneys did not equilibrate with plasma cholesterol until after 7 … (Fig. 3). The curves of Fig. 3 are in keeping with the concept that plasma cholesterol i… precursor of lung and kidney cholesterol. The plasma specific activity curve intersecte… liver curve, but not at its maximum. This result agrees with the general view that a p… plasma cholesterol originates in other tissues than the liver. There is a similar situ… between the specific activity curves of liver free cholesterol and plasma free chole… (Fig. 4 a and b) suggesting that it is the free rather than esterified plasma cholesterol… originates in other tissues.

The specific activity curves of Fig. 4 a are in accordance with the idea that free chole… is the precursor of erythrocyte cholesterol. The specific activity curve of plasma este… cholesterol intersected that of liver esterified cholesterol at its maximum (Fig. 4 a an… which can be interpreted to indicate a direct precursor-product relationship between … and plasma esterified cholesterol in the rat.

The radioactivity reappearing in plasma was associated with lipoproteins isolate… ultracentrifugation (Fig. 5). The specific radioactivity of the low and very low density … protein cholesterol followed closely that of the red blood cell cholesterol, whereas the sp… activity of the high density lipoprotein cholesterol was always clearly higher than th… the other lipoproteins during the rising part of the specific activity curve. Maximum sp… activity was reached in 5–10 h, always earlier in HDL than in [illegible] lipoprotein…

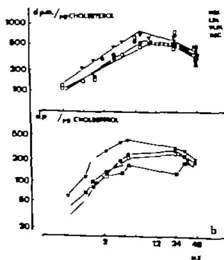


ı Specific radioactivity of free and esterified cholesterol of liver and plasma after injection of
...late ³H-cholesterol. In expt) ³H-cholesterol (20 μCi) was given to 13 rats, in expt. b) ³H-cholesterol
Ci) was given to 8 rats. FC = free cholesterol EC = esterified cholesterol, RBC = red blood cell choles-

...librium of cholesterol specific activity between different lipoproteins was achieved in
48 h.

...parison of the kinetics of particulate and chylomicron cholesterol

...lomicron cholesterol has been shown to be removed from the circulation by the liver
...enchymal cells (Nilsson and Zilversmit 1971), which in the present study appeared
...ponsible for the uptake of particulate cholesterol as well. Therefore the kinetics of choles-
...l in these two forms were compared. Rats were given radioactive ³H-cholesterol by



... 3. Specific radioactivity of plasma lipoprotein
... red blood cell cholesterol after injection of
...rticulate cholesterol H-cholesterol (40 μCi)
... given to 3 rats. b. ³H-cholesterol (25 μCi) was
...en to one rat. Lipoproteins were fractionated
...th swing-out flotation technique (30 000 rev./min,
... 000 g 6 h).

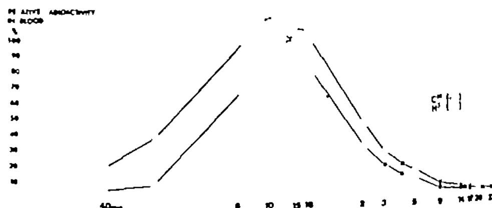


Fig. 6. Relati e radioactivity of blood after i.v i jection of particulate ⁴C-cholesterol and after ... feeding of H-cholesterol. Values are expressed as per cent of calculated ma imum values ³H-chol (25 μCi in olive oil) was given to 7 rats by gastric i tubation and within 30 min ⁴C-cholesterol ... administered i v

gastric intubation and after 30 min ⁴C-cholesterol in particulate form was inject... The resulting radioactivity curves were closely similar in shape (Fig. 6), although the ... micron curve followed about 6 h behind the curve of particulate cholesterol. Th... difference between the curves may be due to the time needed for the absorption of ... terol from the gastrointestinal tract (Zilversmit and Hughes 1974) and for the con... of the chylomicrons into remnant particles (Redgrave 1970). Together with the aut... graphic findings the present results suggest that the mechanism of removal of part... cholesterol from the circulation may be similar to that of chylomicron cholesterol ... *et al* 1969)

Discussion

It has been shown previously that the radioactive particulate cholesterol cleared fr... blood is found in the liver (Avigan 1959 Nilsson and Zilversmit 1972). In the presen... more than 90 per cent of the dose accumulated in this organ (Fig. 1). The short hal... the cholesterol in the blood indicates that the uptake of the radioactivity by the li... rapid. After 1 5 min about 50% and after 5 min 75% of the dose was found in the liv... which blood had been removed by perfusion. This resembles the rapid uptake by ... of cholesteryl esters of the chylomicron remnants (Redgrave 1970) of which 40 ... found in the liver after one min and 92% after 10 min There is also uptake by the ... cholesteryl esters artificially incorporated into rat plasma lipoproteins (Goodm... Lequire 1975) the maximum uptake is 58% of the dose

All the injected particulate cholesterol was in the unesterified form 1 / min a... injection 4 1% of the dose was already found esterified in the liver It is possible tha... of the dose was esterified in blood before reaching the liver but a more likely explan... that the esterification took place in the liver The initial rapid esterification slowed ... 5 min, whereafter a plateau was maintained for 1 h (Fig. 1 b). During this time 5 4–... the dose was in the form of liver esterified cholesterol.

In the radioautographic studies of liver cells by both frozen and smear techniqu...

onal grains were seen over the Kupffer cells (Fig. 2). The radioactivity was associated y with the parenchymal cells already 1.5 min after the injection. It is unlikely that the t observations could be accounted for an artifact, because the tissues were fixed diately after killing the animals. The results, together with the kinetic data (Fig. 6) ts that the handling of cholesterol dispersion in the liver resembles that of chylomicron nts (Stein *et al.* 1969), except that the remnant cholesterol is mostly esterified and is lyzed in the liver Since 6 per cent of the radioactive dose in the present studies was d in the spleen, it is possible that some types of particles were caught up by the reti- ndothelial cells. The distribution of radioactivity in the spleen was not studied in detail, gh.

though labeled cholesteryl esters in emulsified chylomicron lipid and in intact chylo- ons is taken up by the parenchymal cells of rat liver (Nilsson and Zilversmit 1971), on and Zilversmit (1972) found by scintillation counting that the liver Kupffer cells ted by pronase digestion contained the greatest part of the injected particulate choles- Pronase is known to digest the parenchymal cells but not Kupffer cells (Mills and er-Franklin 1969). Since the Kupffer cells are phagocytozing cells, it is possible that activity located primarily in the parenchymal cells was ingested by the Kupffer cells n the parenchymal cells were disintegrated

n the basis of the present results it cannot be concluded, whether all of the particulate esterol entered the liver parenchymal cells or whether some particles remained on the surface. Stein *et al* (1969) have proposed that chylomicron cholesteryl esters are quickly od by the liver parenchymal cell surface and are only slowly transferred into the cell. also possible that pools of differing specific activities were created intracellularly It been suggested by Sodhi and Kudchodkar (1973 a) that separate metabolic pools would t in the liver for cholesterol originating in the intestines and synthesized *de novo* by the r on the one hand and for cholesterol reaching the liver as a part of plasma lipoproteins the other Therefore, caution is required in the interpretation of the kinetics of particulate lesterol re-entering the circulation.

t is apparent that the liver was the chief source of the radioactive cholesterol re-entering plasma At this stage, the radioactivity was associated with the plasma lipoproteins g. 5). Two major mechanisms for the incorporation of liver cholesterol into the plasma oproteins can be considered (a) Particular cholesterol that has entered the liver paren- ymal cells is incorporated into lipoproteins synthesized by these cells. Because cholesterol thesized *de novo* by rat liver first appears in the very low density lipoprotein fraction obelm *et al.* 1963 Gidez *et al.* 1967), it is this lipoprotein that should have had the highest cific activity in the present studies. Since the high density lipoproteins had the highest cific activity the mechanism considered above does not alone explain the reappearance f particulate cholesterol into the circulation, although it may account for a part of it.

(b) The liver parenchymal cells may have mechanism of excretion of cholesterol similar that of cells of peripheral tissues. There is experimental evidence obtained with mam- alian cells in culture that the high density lipoproteins would selectively function as ac- ptors of cholesterol from the cells (Bates and Rothblat 1974, Stein *et al.*, 1976) This con- pt is in agreement with the present observation that the radioactivity first appeared in the



Fig. 6. Relative radioactivity of blood after i v injection of particulate ¹⁴C-cholesterol and the same feeding of ³H-cholesterol. Values are expressed as per cent of calculated maximum then ³H-chole (25 μCi in olive oil) was given to 7 rats by gastric intubation and within 30 min ¹⁴C-cholesterol (5 μCi administered i v

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Discussion

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plasma high density lipoproteins (Fig. 5) However it is also possible that there w exchange of cholesterol between the lipoproteins and cholesterol particles still pres the cell surface By whatever mechanism the high density lipoproteins acquired their active cholesterol the curves in Fig. 5 suggest that this cholesterol could in its turn b able to function as a source for cholesterol of the other lipoproteins.

According to Fig. 4 plasma esterified cholesterol did not appear to acquire all its activity from plasma free cholesterol and obviously a large fraction of esterified chol was derived from the liver This is in agreement with the report of Portman and Sc (1957) in cebus monkey that particular cholesterol reappears in blood as both free chol and cholesteryl esters. There is a precursor product relationship between the cur liver esterified cholesterol and plasma cholesteryl esters (Fig. 4), but any conclusion the proportion of plasma cholesteryl esters originating from the liver are hampered inability to know the homogeneity of the liver cholesterol pools. According to Sw Law (1971) most of plasma esterified cholesterol in the rat is derived from the liver e cholesterol whereas the results of Gidez *et al* (1965) suggest that the cholesteryl e very low density lipoproteins but not those of the high density lipoproteins origi the liver of the rat The presence in rat plasma of lecithin cholesterol acyl transferase *et al* 1974 Stokke 1974) suggests that not all esterified cholesterol is contributed liver Studies in humans (Barter 1974) suggest that the formation of very low dens protein cholesteryl ester takes place by esterification of free cholesterol perhaps in t and in the plasma.

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equally distributed in the two fibre types of human muscle (Piehl *et al.* 1974), judging histochemical analysis, thereby indicating a similar glycogen restorative capacity. Owing to this discrepancy between glycogen breakdown and glycogen storage capacity, the manner in which muscle fibre composition influences the former variable, the study of glycogen phosphorylase and glycogen synthetase enzyme activity in human skeletal muscle was thought to be of interest. This study was performed by means of comparative investigations of the two enzymes in homogenates of skeletal muscle samples with mixed fibre composition and in individual fibre types.

Subjects and Methods

Enzyme studies in muscle homogenates were performed on skeletal muscle biopsies taken at random from 10 young, healthy physical education students at rest. The studies of individual fibre types were made on additional biopsies taken from 4 of these subjects. Age, height and weight averaged 23 (range 20–25) years, 179 (range 169–175) cm and 72 (range 72–73) kg respectively. Maximal oxygen uptake averaged 4.2 (range 3.6–4.7) l min^{-1}. Muscle fibre composition varied among the subjects and averaged 54 (range ...) slow twitch fibres.

Muscle samples were taken from the vastus lateralis employing the needle biopsy technique (Bergström ...). The subjects were resting at the time. They ate an uncontrolled mixed diet and carried out a physical training programme 24 h before the expts. Muscle samples were immediately frozen in liquid nitrogen and stored at −80°C ... immediately analyzed.

Determinations from whole biopsy specimens. Muscle fibre composition was determined according to ... *et al.* (1972) by means of identification of myofibrillar ATPase activity in individual fibres after preincubation at pH 10.3 (Padykula and Herman 1955). The fibres were classified as either slow twitch (ST) or fast twitch (FT).

Glycogen content, expressed as glucose units (Karlsson 1971), and glycogen phosphorylase ($a+b$) activity (... *et al.* 1977) were determined with fluorometric methods originally described by Lowry and Passonneau (1973). Glycogen synthetase activity was measured according to the method of Thomas *et al.* (...) as described by Piehl (1974). Since the biopsies for enzyme analysis were obtained on different days, the glycogen content was determined twice.

Determinations from individual muscle fibre types. For determinations from individual fibre types, biopsy samples were freeze-dried and single muscle fibres dissected under a light microscope at room ... temperature and humidity were controlled and kept constant (Essén and Henriksson 1974). Each single ... was typed (staining for myosin ATPase at pH 10.3) and analyses were made on batches (10–70 μg ... weight) of ST and FT muscle fibres respectively. Glycogen determination was carried out employing the method presented above, although the glycogen hydrolyzation procedure was performed using a smaller volume and in a capillary tube (for details see Lowry and Passonneau 1973, Essén and Henriksson 1974). The enzyme activity determinations were also carried out as described above, although the homogenates were prepared somewhat differently. The glycogen phosphorylase ($a+b$) activity determinations were made on homogenates of pooled ST and FT fibres respectively, the dilution ratio corresponding to 1 ... w/v. The dilution ratio for glycogen synthetase activity was 1 ... w/v.

Results

A linear increase was observed in glycogen synthetase activity (I + D) compared to glycogen phosphorylase ($a+b$) activity (Fig. 1). A similar relationship was found for the 4 samples obtained for further study of enzyme activity and glycogen content in individual fibre types (Table I).

Glycogen synthetase (I + D) activity was found to range from 7.6–11.9 μmol · g (dw)$^{-1}$ · min^{-1} whether analyzed in pooled ST (mean value 9.5 μmol · g (dw)$^{-1}$ · min^{-1}) or FT fibre

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μmol · g (dry weight)$^{-1}$ · min

Acta physiol scand. 1977 100 210-214
From the Department of Physiology Gymnastik och Idrottshögskolan, Stockholm, Sweden

Glycogen Synthetase and Phosphorylase Activity in Slow and Fast Twitch Skeletal Muscle Fibres in Man

By

KARIN PIEHL and JAN KARLSSON

Received 15 December 1976

Abstract

PIEHL K. and J KARLSSON *Glycogen synthetase and phosphorylase activity in slow and fast twitch skeletal muscle fibres in man* Acta physiol scand. 1977 100. 210-214

Glycogen synthetase (I + D) and phosphorylase (*a* + *b*) activity was determined in human skeletal muscle biopsies with different muscle fibre composition and in dissected and pooled batches of the two main fibre types slow twitch (ST) and fast twitch (FT), respectively. Glycogen synthetase (I + D) activity remained unchanged as the per cent of FT fibres increased but phosphorylase (*a* + *b*) activity was found to increase. A similar activity pattern was found in dissected and pooled FT fibres as compared to ST fibres, i.e. the same glycogen synthetase activity but heightened phosphorylase activity

The rate of carbohydrate utilization increases with increasing work load, and a pronounced increase in glycogen depletion is seen when intensity approaches maximal and supramaximal work levels (Saltin and Karlsson 1971 Gollnick *et al* 1974). It has been suggested that the major source for carbohydrate metabolism at heavy exercise loads is muscle glycogen (for ref see Saltin and Karlsson 1971) However repletion of glycogen in the muscle cell following exercise depletion has been shown to be a relatively slow process compared to glycogen utilization despite an adequate carbohydrate intake (for ref see Piehl 1974).

Even individual muscle fibre composition, in addition to exercise intensity was recently shown to be of significance for the rate of glycogen depletion. Juhlin-Dannfelt (1976) showed that depletion was greater in muscles rich in type II or fast twitch (FT) muscle fibres than in slow twitch (ST) fibres. This finding was in conformity with the more pronounced glycogenolytic profile found for FT muscle fibres (Gollnick *et al* 1974 Sjödin 1976).

Glycogen breakdown is achieved by release of glucose residues from the glycogen molecule by the enzyme glycogen phosphorylase (E.C.2.4 1 1) This enzyme is activated by conversion of the enzyme from an inactive form (phos *b*) to an active form (phos *a*) additional activation is produced by phos *a* in the presence of elevated 5 AMP levels (for ref see Karlsson *et al* 1977)

The enzyme glycogen synthetase (E.C.2.4 1 11) which also exists in two interconvertible forms, the D-form (dependent of G-6-P) and the I form (independent of G-6-P) (Danforth 1965) is the rate limiting step for glycogen synthesis in the muscle cell This enzyme

II Glycogen phosphorylase ($a+b$), glycogen synthetase (I+D) activities, % of I-form in total activity and glycogen content in dissected and pooled ST and FT fibres respectively. The values are related to dry weight (dw).

Fibre types investigated	Glycogen phosphorylase ($a+b$) µmol g (dw)$^{-1}$ min^{-1}	Glycogen synthetase (I+D) µmol g (dw)$^{-1}$ min^{-1}	% of I-form in total activity
slow twitch fibres	1.54	9.5	22
	1.57	9.6	18
	1.78	10.7	15
	1.71	11.9	19
	1.65	10.4	19
fast twitch fibres	2.23	7.6	13
	2.47	11.2	20
	2.31	9.8	16
	2.73	9.2	16
	2.44	9.5	16

data ere based in part on the same material, with the exception of data for the 4 more nsively studied subjects. However the data even here followed the same pattern.

lycogen synthetase (I D) activity was not related to muscle fibre composition, al-ugh there was a relationship between synthetase and phosphorylase activities. This might n that the two enzymes are regulated independently Further support for this view was rided by the data on pooled FT and ST muscle fibres. Glycogen phosphorylase ($a+b$) vity as considerably higher in FT fibres, whereas glycogen synthetase activity was roximately the same in both fibre types.

: might be argued that the freeze-drying process could inhibit enzyme activity thereby uring the relationships in pooled f bres as compared to the homogenate of whole speci-s. The glycogen content ratio in dried/wet samples was on the order of 5 which is similar he value expected from water content ranging from 20–25% (Karlsson 1971). The responding ratios for glycogen phosphorylase (a b) and glycogen synthetase (I+D) vities were approximately 3.5 and 2 respectively In other words, almost 50% of the cogen synthetase (I D) activity was lost, whereas phosphorylase only displayed a minor line of the same magnitude as declines found for other enzymes following similar inter tion (Thorstensson 1976). Essén *et al.* (1975) reported striking activity losses similar to se for *synthetase* in the case of phosphofruktokinase (PFK ratio 1.8) and succinate ydrogenase (SDH ratio 2.6). It is possible that the explanation offered by Essén and vorkers, based on a time-dependent deterioration of the freeze-dried enzyme, is the most nant, although alternative explanations, based on .r the differences in isozyme patterns and in enzymes, can be suggested.

The main question is whether deterioration processes are qualitatively and quantitatively ilar in both fibre types. Assuming that this is the case, the present data would only indicate triking difference between the fibre types in respect to glycogen phosphorylase ($a+b$) ivity

study as supported by grants from the Swedish Medical Research Council (B76-04X-04251-03) and ...h C ...sh Sports Federation.

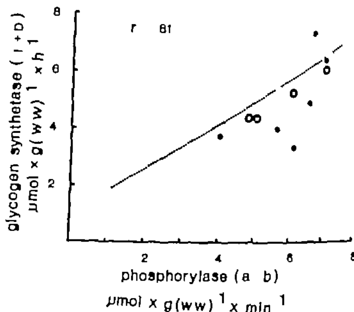


Fig. 1 Glycogen synthetase (I + D) activity in relation to glycogen phosphorylase (a b) activity in the same muscle. The bold dots correspond to the entire material of mixed samples and the open stars a subsample (n = 4) on which analyses were made of slow twitch and fast twitch muscle fibre respectively

(mean value 10 4 μmol × g (dw) min) samples respectively In both fibre ty I-form averaged 19% (ST fibres) and 16% (FT fibres) respectively (Table II). G phosphorylase ($d+b$) activity ranged from 1 54–2.73 μmol × g (dw)$^{-}$ when analyze and FT fibres separately In contrast to the case for glycogen synthetase (I + D) t there was a clearcut difference in enzyme activity between ST and FT fibres, and amounted to 1 65 and 2.44 μmol g (dw) min respectively

In the analyses of pooled fibres, glycogen content ranged from 572–811 μmol g Higher values were obtained in ST fibres in all individuals except one. The mean le 748 compared to 650 μmol g (dw) in FT fibres (Table II).

Discussion

Glycogen phosphorylase ($d+b$) activity has previously been shown to increase w muscle fibre composition (Gollnick *et al* 1974 Karlsson *et al* 1977 Sjödin 1976). Th

TABLE I. Glycogen phosphorylase ($d+b$), glycogen synthetase (I + D) ctivities, % of I-form in total and glycogen content in whole biopsy samples. The values re related to wet weight (ww)

% FT fibre	Glycogen phosphorylase ($d+b$) μmol × g (ww)$^{-}$ × min^{-}	Glycogen μmol g (ww)$^{-}$	Glycogen synthetase (I + D) μmol g (ww)$^{-}$ min	% of I-form in total activity	Glyco μmol g (ww)
41	0 49	113	4 4	14.5	152
53	0.61	121	5.3	14 7	139
56	0 48	132	4 4	15.9	130
75	0 71	168	6.1	13 7	123
Mean	0.57	134	4 6	14 7	136

Glycogen phosphorylase (*A*+*b*), glycogen synthetase (I+D) activities, % of I-form in total activity and glycogen content in dissected and pooled *ST* and *FT* fibres respectively. The values are related to dry weight (dw)

Fibre types investigated	Glycogen phosphorylase (*A*+*b*) µmol g (dw)$^{-1}$ min^{-1}	Glycogen synthetase (I+D) µmol g (dw)$^{-1}$ min^{-1}	% of I-form in total activity
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Acta physiol. scand. 1977. 100. 215–220

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Comparative Effects of Adrenaline and Felypressin (Octapressin) on Consecutive Sections of the Vascular Bed in Canine Adipose Tissue

By

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Received 16 December 1976

Abstract

HER, E., L. OLGART and B. GAZELIUS. *Comparative effects of adrenaline and felypressin (octapressin) on consecutive sections of the vascular bed in canine adipose tissue.* Acta physiol. scand. 1977. *100.* 215–220.

flow and tissue volume were recorded in the isolated canine subcutaneous adipose tissue, enclosed plethysmograph. Adrenaline and felypressin (octapressin) were infused intra-arterially at doses pro- blood flow reduction of approximately 60 %. Adrenaline (4.6–23 ng/min) caused an partial reduc- n tissue volume, indicating constriction of capacitance vessels. Octapressin (0.46–9 ng/min) had fect on tissue volume. Neither adrenaline or octapressin caused appreciable filtration or absorption, sting that the pre- to postcapillary resistance ratio remained unchanged. In contrast to sympathetic lation and noradrenaline, adrenaline significantly reduced the capillary filtration coefficient (CFC) the change in CFC being related to the flow reductions, while octapressin did not reduce CFC cantly. On the basis of these results it is suggested that the constrictor effect of adrenaline on pre- lary sphincter sections and on capacitance vessels is greater than that of octapressin in doses producing ar flow reductions.

h adrenaline and octapressin reduce the absorption of local anesthetic solutions and ong the anesthesia (Berde *et al.* 1961, Åkerman 1966, Berling 1966). These findings cate similar constrictor effects of adrenaline and octapressin on precapillary sections ling to a reduced capillary surface area.

However, other observations suggest that adrenaline and octapressin may have different cular effects. For example, it was shown in the cat that anesthetic solutions with adrena- e injected into the oral mucosa caused a marked reduction of blood flow in the tooth, along to a total abolition of intradental sensory nerve function (Olgart and Gazelius 77). Similar injections with octapressin were without such effects. One possible explana- n for these differences between adrenaline and octapressin may be found in earlier ob- vations by Altura *et al.* (1965), showing that octapressin had a weaker constrictor effect arterioles than adrenaline following topical application to the rat mesenteric vasculature. heir results indicate that adrenaline has a comparatively stronger constrictor action on

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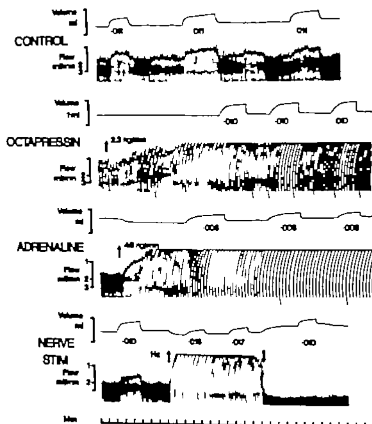
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1 Effects of intra-arterial infusion of octapressin, adrenaline and sympathetic stimulation on blood flow, tissue volume and CFC on an isolated subcutaneous adipose tissue preparation (weight 40 g). CFC repeatedly determined by elevation of the venous outflow pressure by 15 cm H_2O. These values for each procedure are given in the volume recording

during the steady state phase of drug action (Fig. 1). Octapressin produced only a very small initial volume decrease in 2 expts., with no further change of this parameter. Sympathetic stimulation at 3 to 4 Hz caused a fairly marked initial decrease in volume (0.5 ml/100 g) without any further change in volume (Fig. 1).

TABLE 1 Effect of adrenaline and octapressin on CFC and blood flow. The values given are the mean responses. Figures within parentheses denote ±1 S.E.

		CFC ml/min/mmHg/100 g	Change in flow
Control	41	0.0141 (±0.0007)	
Adrenaline	18	0.0101 (±0.0012)	61
Octapressin	24	0.0140 (±0.0011)	61

precapillary resistance vessels Therefore, local injections of adrenaline may reduce flow to more distant peripheral tissues, while injections of octapressin have no such The differences however may also be explained by another mechanism. Octapressin much larger molecule than adrenaline and may therefore have a more restricted capacity Thus octapressin may only have very local effects while adrenaline can reach distant vessels from the injected depot

The purpose of this investigation was to make a more detailed study of the effects octapressin and adrenaline in order to further elucidate their vascular actions.

Methods

7 female mongrel dogs were anesthetized with sodium pentobarbitone, 30 mg/kg i.v. supplemented necessary

The inguinal subcutaneous adipose tissue was prepared according to the method of Rosell (1966 adipose tissue was completely isolated from all surrounding tissue except for the artery vein and which was cut. Heparin 2 500 IU/kg was administered i.v. to prevent clotting and the adipose tissue was then cannulated and perfused with blood from the femoral artery via an exteriorized loop a silicon-filled drop recorder The vei was also cannulated with long, wide-bore plastic tubing end could be raised or lowered to change venous pressure. The venous blood was returned to the The adipose tissue was then placed in a water tight plethysmograph filled with Tyrode solution described by Öberg and Rosell (1967).

Tissue volume was recorded n a Grass polygraph via a volume recorder connected to the plethysmograph. The volume recorder was calibrated several times during the expt. Adipose tissue blood flow carotid blood pressure (via Statham pressure transducer) were also recorded on the Grass polygraph

Intra-arterial infusion (0 04–0.2 ml/min) were given via side-arm in the arterial circuit. Drugs made up in saline and kept cooled on ice. Absorbic acid (20 μg/ml) was used in the adrenaline The rat of drug infusion was designed to give a decrease in blood flow of approximately 60%. pressure was unchanged by drug infusion.

In 2 expts., the sectioned nerve t the adipose tissue was stimulated via bipolar silver electrodes at 2 ms and 12 V for 10–12 min, using Grass stimulator

Resting blood flow ranged from 1 8 ml/min/100 g to 1..1 ml/min/100 g (mean 7 6 ml/min/100 g tissue). This is similar to previously reported values in this tissue (Öberg and Rosell 1967) Adipose weights were 70 to 50 g (mean 35 g). I general, the smaller ad pose tissues had higher flow rates than larger tissues.

Capillary filtration coefficient (CFC) was determined by raising the venous ca ula by 15 cm, in an increase in capillary hydrostatic pressure, according t the method of Cobbold et al. (1963). Increase in venous pressure causes n initial increase in volume, assumed to be due to distension of tance vessels, followed by a slow increase in volume, due t net outward filtration of fluid (Folkow 1963). CFC, expressed as ml fluid f ltered/min/mmHg/100 g, was determined as described by Cobbold et al. (1963) and F lkow et al. (1963). Data are expressed as mean ± 1 S.E. (number of observations

Results

Blood flow Octapressin was infused in 6 expts. at a dose range of 0 46 to 9.2 ng/min ca a mean reduction in blood flow of 61%, compared to control Adrenaline infused at a range of 4 6 to .3 ng/min, also reduced blood flow by 61% (6 expts.) Data where blood was reduced by less than 40% or more than 80% have been excluded.

Tissue volume Adrenaline infusion usually produced a small rapid reduction in volume indicating a decreased regional blood content presumably due to constriction veins. Following the initial decrease (0.3 ml/100 g) there was no further change in v

respect. The reduction of CFC by adrenaline is interesting in view of previous findings ng that both noradrenaline and sympathetic stimulation reduce the capillary surface ut increase CFC in this and other vascular beds (Cobbold *et al.* 1963 Öberg and Rosell Fredholm *et al.* 1970 Järhult 1971) This effect of sympathetic stimulation on CFC een suggested to be due to increased vascular permeability mediated by -adrenergic tors (Fredholm *et al.* 1970, Linde *et al.* 1974). The present results with adrenaline st that in the subcutaneous adipose tissue the constrictor effect of adrenaline on pre lary sphincter sections is greater than that of noradrenaline and sympathetic stimulation. e effects on the capacitance function can be deduced from the tissue volume changes. naline almost invariably caused a constriction of capacitance vessels as shown by the l reduction in tissue volume at the start of the adrenaline infusion. Sympathetic stimula- was even more potent than adrenaline in this respect. Octapressin, on the other hand, almost no effect on this parameter suggesting that it has no effect on capacitance vessels. e results are in contrast to previous findings in perfused skin of the cat showing that pressin caused a marked and sustained constriction of capacitance vessels (Cerletti l 1963). They used rapid intra-aortic injections of high doses of octapressin, causing nounced systemic pressure effects which were not obtained in the present study differ s that may explain the contradictory results.

he effects of different drugs on postcapillary resistance vessels, as distinct from capaci- e essels are more difficult to assess in this preparation. However some information be obtained from the slow phase of volume changes, which reflects outward or inward ation. Neither adrenaline or octapressin caused pronounced outward or inward filtra implying that the mean hydrostatic capillary pressure was essentially unaltered. A slight dency to outward filtration by adrenaline was noticed in some experiments. Therefore, pre to postcapillary resistance ratio seems unaltered by both drugs, indicating that striction of postcapillary vessels occurs, with adrenaline having slightly greater con- ctor effect since some filtration occurred.

The similarity in effects of adrenaline and octapressin on the resistance in pre and post illary sections are in contrast to the findings by Altura *et al* (1965) in the rat mesentery culature. They showed by topical application of the substances that octapressin was a tent constrictor of small venules or postcapillary resistance vessels with a relatively weaker nstrictor effect on arterioles compared t adrenaline. The discrepancy in results may be plained by the different routes of administration and different diffusion properties of the bstances. Octapressin is much larger molecule than adrenaline and may have slower and ore restricted diffusion capacity than adrenaline. Such properties of the substances may so explain the different effects of adrenaline and octapressin on dental pulp blood flow llowing submucosal injection as was recently shown by Olgart and Gazelius (1977). Adren- line reduced pulp blood flow while octapressin had no such effect. Intraosseous vessels applying the dental pulp may thus not be reached by an effective concentration of octa ressin from the depot. This dissimilarity between adrenaline and octapressin may also artly explain the relatively long latency in constrictor response following infusion of octa ted effect after infusion as compared to adrenaline in the present

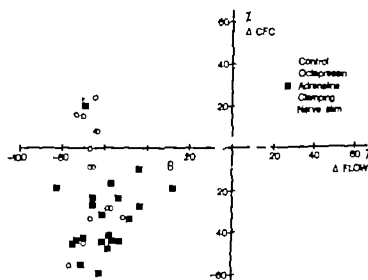


Fig. 2. Data from experiments showing change in CFC expressed as percent of control plotted against the corresponding change in blood flow, expressed as percent of control

Capillary filtration coefficient Adrenaline, at infusion rates giving a 40 to 80 % reduction in blood flow significantly ($P < 0.005$) reduced the CFC (Table I), while octapressin did not alter the CFC

Since there was some variation in the values of CFC between different expts the CFC has also been expressed as percent change in CFC. The results of all expts. are summarized in Fig. 2, showing per cent change in CFC plotted as a function of change in blood flow. Adrenaline reduced CFC in 5 out of 6 expts. the change in CFC being related to the change in blood flow The average reduction of CFC by adrenaline was 34 %, while octapressin reduced CFC by 13 %.

Time course of vasoconstriction In general, the effects of octapressin were rather slow in onset, taking up to 20 min for steady state vasoconstriction to occur and were even slow in disappearing after the infusion (at least 20 min) The time course of adrenaline vasoconstriction was rapid both in onset and disappearance.

Discussion

The present study is an attempt to compare the effect of adrenaline and octapressin on different sections of the vascular bed in adipose tissue.

The doses of octapressin and adrenaline were chosen in order to give approximately the same reduction in blood flow so at these doses it is probable that they have similar effects on the precapillary resistance vessels.

The most significant result of this investigation is the finding that adrenaline reduced CFC by 34 %, whereas octapressin had no significant effect. Since CFC is a function of capillary surface area and permeability a change in either or both of these parameters will influence CFC. So far there is no experimental evidence that these vasoconstrictor substances have any effect on capillary permeability Adrenaline therefore seems to decrease capillary surface area by constriction of precapillary sphincter sections while octapressin is

Acta physiol. scand. 1977 100 221–230

From the Institute of Physiology University of Oslo, Norway

Small Airway Constriction and Closure after Induced Intravascular Platelet Aggregation

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Received 22 December 1976

Abstract

J and A. HAUGE. *Small airway constriction and closure after induced intravascular platelet aggregation.* Acta physiol. scand. 1977 *100* 221–230

of this study as to investigate the function of the peripheral airways after intravascular platelet ion induced by infusions of collagen in open chest anaesthetized cats, ventilated with constant ume Lung compliance as examined under static conditions (stat C_L) and under dynamic condi- n C_L) at ventilation frequencies of 5, 24 and 50/min. In the control situation dyn C_L 24 was approxi- 0 of stat C_L. Collagen infusions resulted in pronounced frequency-dependence of lung com- When dyn C_L 24 had decreased by approximately 40 reduction in stat C_L could also be detected. yn C_L 24 decreased even more concurrent reduction in stat C_L as evident. These findings in- hat the initial event after induced intravascular platelet aggregation is small airway constriction. ore pronounced changes airway closure and reduction in lung volume occurs. We suggest that these al changes in peripheral airways result in the impaired gas exchange known to occur after intra- r platelet aggregation.

respiratory insufficiency will sometimes evolve in patients who are critically ill after ve trauma, shock or septicemia, although neither chest trauma nor preceding cardio- nary disease has been present. This particular lung dysfunction, sometimes called raumatic lung insufficiency or shock lung, is characterized by severe, arterial hypoxemia o disturbances in the ventilation–perfusion ratio with intrapulmonary shunting of blood b 1969 Wardle 1974).

ere is substantial evidence indicating that pulmonary microembolism due to dissemi- l intravascular coagulation and/or intravascular platelet aggregation is key factor in evelopment of this syndrome (Bo and Hognestad 1971 Blaisdell 1974 Wardle 1974 nn 1975 Saldeen 1976). Pulmonary insufficiency will also develop when longlasting vascular aggregation of blood platelets is experimentally induced in cats (Vaage 1976 b). nother reason for focusing attention on blood platelets is the fact that their activation aggregation appear to be causative factors in the bronchoconstriction and pulmonary spasm observed after embolization of the lungs with blood clots (Thomas *et al.* 1965 *et al.* 1970) or with nonbiological agents such as barium sulphate (Bo Hognestad and

This study was supported by the Swedish Medical Research Council (Grant No B75-X4-1(Karolinska Institutet. Elizabeth Burcher was a recipient of a Fellowship from the Swedish Nat As sociation against Heart and Chest Diseases. We would like to thank Miss Lotta Malmström and Margareta Stensdotter for skilful technical assistance.

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I The experimental protocol showing time table of lung inflations, measurements of lung compliance, and ventilation frequency in group B. At the arrow an infusion of collagen (0.5 ml) is given over 1 min. All times refer to the start of collagen infusion, which is called zero time.

..tion ..ncy (..hs/min)			24	5	24	50	24			4	↓	24	5	4	90	24
..ompliance ..red			+	+	+	+				+		+	+	−	−	+
..nflation	+	+						+	+							
..min)	25	−20	−15	−14[??]	−14	−13[??]	−13	−10	−5	−1	0–1	1[??]	[illegible]	[illegible]	3[??]	3[??]

..lung compliance (stat C_L) ..as measured by disconnecting the animals from the respirator for short ..of time. The lungs were then stepwise inflated with air from .. 50 ml syringe starting at .. P_{TP} .. cm H_2O. Each step consisted of .. 10 ml inflation, and the interval between two such steps ..as 5 ..al of 4 consecutive inflations were done. P_{TP} reached .. stable level 1–2 .. after finishing each step of ..on. A volume-pressure ratio ..as calculated for each step. Since these ratios fell on .. straight curve ..as calculated as the mean of the values obtained from the 4 consecutive inflations.

..elet aggregation was induced by .. infusions over 1 min of .. suspension of collagen fibrils as previously described (Vange, Bø and Hognestad 1974; Vange 1976). The collagen suspension was prepared ..scribed by Holmsen (1969).

..perimental protocol. When the surgical procedures were finished the lungs were briefly inflated to .. P_{TP} of 20 cm H_2O by clamping the expiratory tubing. Such lung hyperinflations are performed regu.. 5 and 10 min before measurements of lung compliance in order to counteract airway collapse and to ..e equal volume histories of the lungs before collagen infusion. The animals were left for 15–20 min ..the end of surgery to let the hemodynamic and respiratory variables recorded become stable. 3 different ..rimental groups were used.

..Group A 10 cats ..ere given only one infusion of collagen in order to study the spontaneous time course ..decrease in dyn C_L 4. Measurements ..ere carried out 60 .. before start of the collagen infusion, 105 ..the start of this infusion and then at intervals of 30 .. during the next 2½ min.

..Group B dy.. C.. 5, dyn C.. 4 and dyn C_L 50 ..ere measured before and after collagen infusions. Table I ..s the protocol used before and after the first collagen infusion. Three consecutive collagen infusions ..animals ..ere given 20 min apart. I.. connection ..ith the second and third collagen infusion only the ..ocol from 10 min before the start of collagen infusion and onwards in Table I was used.

..animals did not receive any collagen infusions, but the series of compliance-measurements was ..ated 3 times ..ith intervals of 20 min in order to test the reproducibility of the method. Hyperinflation ..he lungs ..as performed 5 and 10 min before each series of tests, such as in the animals given collagen ..sions. W.. found that lung compliance measured at the same frequencies differed less than 5 per cent ..period of 60 min.

..he control value of dyn C_L 5 ..as set to 100 .. and all other compliance data were related to this one. ..value of dy.. C_L 4 to be compared ..ith dyn C_L 5 was the mean of dyn C_L 24 30 .. before and after ..measurement of dyn C_L 5.

..in roup C dy.. C.. 24 was related to stat C_L before and after .. collagen infusion in 5 animals, using the ..ocole outlined in Table II. The control value of stat C.. ..as set to 100 .. and all other compliance data

..BLE II The experimental protocol showing time table of lung inflations, measurements of lung compliance, and ventilation frequency in group C. A.. the arrow an infusion of collagen (0.5 ml) is given over 1 min. All times refer to the start of collagen infusion, which is called zero time.

..entilation ..equency (breaths min)		4	0	24			24	↓	4	0	24
..ung compliance ..easured											
..nflated											
	20	15	14[??]	14	10	5	1	0–1	1[??]	2[??]	[illegible]

Vaage 1974) Broncho- and pulmonary vasoconstriction will usually cause changes in the ventilation-perfusion ratio with arterial hypoxemia. Such changes are known to occur after pulmonary embolization (Robin *et al* 1960 Levy and Simmons 1975).

When sudden intravascular platelet aggregation is experimentally induced the following lung changes will take place a rise in pulmonary vascular resistance (PVR), a decrease in dynamic lung compliance (dyn C_L) and an increase in non-elastic pulmonary resistance (R_L) (Rådegran 1971 Vaage, Bo and Hognestad 1974).

We believe that further knowledge about the function of small airways following experimentally induced platelet aggregation, might help explain the arterial hypoxemia occurring when platelets aggregate in the circulation The aim of the present work was to study the airway changes underlying the reduction in dyn C_L elicited by induced intravascular platelet aggregation. This was done by studying the effect on lung compliance of changing ventilation frequency A short preliminary report has been presented elsewhere (Vaage and Hauge 1975)

Methods

Cats, weighing 2.2-4.6 kg were used They were anesthetized by i traperitoneal injections of sodium pentobarbitone (Nembutal® Abbott, 30-40 mg/kg)

Ventilation A tracheal cannula was inserted and the cats were given a muscle relaxant (Alloferin® Hoffmann-La Roche, 0.1 ml/kg). Thereafter positive pressure ventilation with a constant volume respirator was started ("Small animal respirat r" model 663 Harvard Apparatus, Mass U.S.A.) A respiratory frequency of 24/mi was used. End-expiratory pressure was kept t +1.5 cm H_2O by means of an expiratory resistor nd the tidal volume (TV) adjusted so as t give pH in arterial blood of 7.40-7.44 at the start of the expt. (pH measured by Radiometer Acid Base Analyser PHM 71 Copenhagen, Denmark) (The tidal volume in cats weighing 3-4 kg, usi g a ventilation frequency of 4/min, is normally 30-45 ml (Vaage, unpublished observations))

Surgical procedure Vascular pressure and flow recordings The sternum was split and the chest opened widely Polyethylene catheters were introduced i to a femoral artery into the pulmonary artery through the right ventricle, and to the left atrium, fo record ng of femoral arterial pressure (P_{FA} with a Statham P23Gb transducer), pulmonary arterial pressur (P_{PA} with Statham P23Db transducer), and left atrial pressure (P_{LA} with Statham P23De transducer) Cardiac output (minus flow through the coronary circulatio) was measured by flowprobe placed around the ascending aorta, and connected to square wave electromagnetic flowmeter ((type 372, Nytron A/S Norway) The pressure and flow parameters were recorded on an 8-channel polygraph (Grass Model 7B Grass Instrument Co Quincy Mass U.S.A.) A polyethylene catheter was inserted int the bd minal aorta through the femoral artery for arterial blood sampling. A catheter was placed n femoral vein for infusion of drugs.

Measurements f tracheal pressure tracheal flow nd tidal olume The tracheal rflow was measured by a screen pneumotachograph designed for small mals (Hewlett Packard). The pressure difference across the screen was measured by differential pressure transducer (Model 70, Hewlett Packard) and calibrated to give airflow The airflow signal was electronically tegrated (Respiratory Preamplifier Model 350-5000 B Hewlett Packard) to give tidal volume. The transpulmonary pressure (P_{TP}), which in open chest conditions equals the tracheal pressure, was measured by another d fferential pressure transducer (Model 270, Hewlett Packard), connected by T t be t the tracheal cann la. Tracheal flow, tidal volume nd transpulmonary pressure were recorded with a Sanborn 6-channel polygraph (Model 3 Hewlett Packard)

D namic lung complianc (dyn C_L) wa calculated as the ratio of the tidal volume to the difference in transpulmonary pressure between the two situations of zero airflow w thin one respiratory cycle (Marshall 1965). This ratio was calculated for 3 consecutive respiratory cycles and the mean value used as the expression of dyn C_L Dyn C_L was measured at ventilation frequencies of 5 24 and 50 breaths per min (dyn C_L 5 dyn C_L 24 and dyn C_L 50 espectively), by cha ging the rate of the respirator The quantity

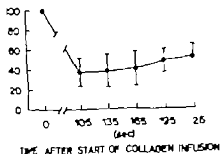


Fig 1

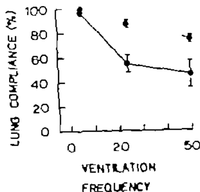


Fig 2

. Time course of the reduction in dynamic lung compliance after 1 min collagen infusion. Zero time ... start of infusion All values are mean ± S.D (= 10).

2. The continuous curve demonstrates the frequency-dependence of lung compliance after infusion of ... (5 infusions in 3 cats). The hatched curve demonstrates the control situation, before the first ... infusions, in the same animals. In this group lung compliance measured under semi-static condi... (5) was not changed by collagen infusions.

...t of ventilation frequency on lung compliance following collagen infusion
...up B and C)

... observations from group B and C were arranged in 2 sub-groups. In one stat C_L was ...hanged and only dyn C_L was reduced, in the other both stat C_L and dyn C_L were reduced ...r collagen infusion. Both true stat C_L and the quasi-static measurement dyn C_L 5 were ...sidered as representative of stat C_L. Reductions of stat C being less than 10 per cent ... the control value were not considered significant.

Reduced dyn C and normal stat C_L (Subgroup 1). This combination was found after 5 ...lagen infusions in 3 cats which all happened to fall in group B. Fig. 2 shows the frequency-...pendence of C_L in these animals, in the control situation and after collagen infusion. ...th dyn C_L 24 and dyn C 50 were reduced by approximately one third while dyn C_L 5 ...mained constant. The most striking reduction of compliance with increasing ventilation ...quency occurred between static conditions and 24 breaths per min.

Reduced dyn C and reduced stat C (Subgroup 2). This combination was found after 10 ...llagen infusions in 4 cats in group B as shown in Fig. 3 Again, the largest reduction of ...ng compliance occurred between static conditions and 24 breaths per min. In this frequency ...terval compliance fell from 80 ± 8 to 37 ± 14 per cent of the control value of dyn C_L 5 ...mean S D).

The 5 expts. in group C also fulfill the criteria of this subgroup. The individual results ...re given in Fig. 4 In these experiments large reductions in dyn C_L 24 sometimes occurred ...ith only moderate changes in stat C However with more pronounced reductions in ...tat C_L the reduction in dyn C_L 24 and stat C_L was almost equal, *i.e.* as stat C_L fell, lung ...compliance became less frequency-dependent.

TABLE III The effect on lung compliance of changing ventilation frequency in 2 groups of ... Lung compliance in each animal is expressed in per cent of the value obtained with ... ventilation frequency All values are mean ± S.D

Number of expts.	Number of measurements	Lung compliance (%)		
		5 breaths/min	4 breaths/min	50 breaths/min
7	11	100	87 6±3	75.2±3 4
		Static	24 breaths/min	
7	11	100	91.5±5 5	

were related to this one. The value of dyn C_L 24 to be compared with stat C_L was the mean of dyn C_L ... 30 s before and after the measurement of stat C_L, again expressed in per cent.

Another 2 animals did not receive any collagen infusions. In these animals the series of compliance measurements was repeated 3 times with intervals of 20 min in order to test the reproducibility of the ... Hyperinflation of the lungs was performed 5 and 10 min before each series of tests, such as in the ... given collagen infusions. Lung compliance measured at the same frequencies differed less than 5% ... one series t another

Results

Intravascular platelet aggregation, is known to cause an increase in P_{PA} an increase ... pulmonary vascular resistance (PVR) a decrease in dyn C_L 24 and concomitantly an increase in airway resistance (R_L) (Rådegran 1971 Vaage, Bø and Hognestad 1974). These ... are transient ones and before any second induction of platelet aggregation, i.e. before ... second collagen infusion, the lung variables had as a rule returned to normal values. ... though the effects of collagen infusions may vary greatly from one animal to another, ... found that 3 consecutive infusions caused similar responses in each animal when the pre... experimental model was used (Vaage, Bø and Hognestad 1974 Vaage 1976 a).

Frequency-dependence of lung compliance in normal cats

In the control situation lung compliance fell slightly when the ventilation frequency ... increased from 0 or 5 to 24 breaths per min (Table III) A further reduction in lung compliance was always observed when ventilation rate was increased to 50 breaths per min.

When the animals were disconnected from the respirator in order to measure stat ... P_{PA} increased from 14.2±1.2 mmHg to 21 8±1 7 mmHg (mean ± S D n = 11) over ... period of measurement. This pressure rise was most likely caused by transient pulmonary vasoconstriction elicited by alveolar hypoxia in connection with stat C_L registration. Dyn C_L 24 measured immediately afterwards while P_{PA} was still increased, was similar ... dyn C_L 24 obtained just prior to the measurement of stat C_L (difference < 5%).

Alteration in dyn C_L 24 with time after collagen infusion (Group A)

The maximal decrease of dyn C_L 24 was usually observed 105 to 135 s after the start of collagen infusion (Fig. 1) During the next 3 min dyn C_L 24 rose gradually following a ... rate of recovery All the measurements of lung compliance at various ventilation frequencies were performed in this time interval (between 105 and 225 s after ... infusion

d considerable obstruction may be present in them with little effect on pulmonary (Brown *et al.* 1969). However despite their small contribution to overall flow ; whether or not small airways are patent will have a major influence on gas distri-d on lung distensibility. For example, if 50 per cent of bronchi at a given level of g were obstructed, total airway resistance might increase only a few per cent, but ensibility would be reduced by about half. Woolcock, Vincent and Macklem oked for abnormal distensibility in humans with mild bronchitis and found that ormality was indeed present, particularly when the patients were asked to breathe As pointed out by Mead (1970) such a frequency-dependence was to be expected he presence of partial obstruction all abnormalities of distension tend to disappear sufficient time. Narrowing of small airways may therefore go undetected, if low y compliance and conventional airway resistance are the only lung-mechanical ployed.

y dynamically measured compliance (dyn C_L) regardless of ventilation frequency e compared with true static compliance (stat C_L). Since however determination C_L caused hypoxia and, most likely a pulmonary vasoconstriction, stat C_L 5 has in the present test-series been used instead. Dyn C_L 24 was of particular interest since e ventilation frequency employed normally in earlier works on lung effects of collagen platelet aggregation from our laboratory. Dyn C_L 50 was included since it has been d that only at such high ventilation-frequencies will obstruction in small airways be l with sufficient degree of certainty (Mead 1970).

: is no general agreement on where to draw the borderline between normal and patho-frequency dependence of lung compliance. The normal adult human lung has airways ently differing lengths and diameters so it seems unlikely that the time constants of alveolar units are uniform. Since, however the peripheral resistance is so low the time-constants will all be very short. Consequently non-uniformity of time constants st to considerable degree without major differences between static and dynamic nce. Grimby *et al.* (1968) found that static and dynamic compliance were similar uencies up t 100 breaths per min in most adult normal humans. Later Woolcock 1969) suggested that dyn C values at high entilation rates (60–120/min) were ab-l if they amounted to less than 80 of the static value. Chiang (1971) propounded hen dyn C decreased from quiet breathing by more than 20 per cent at a respiratory 80–100 breaths/min this might be indicative of small airway obstruction. Cat lungs, er should rather be compared with the lungs of children, which have smaller airway tions and thus higher peripheral airway resistance (Hogg *et al.* 1970). Therefore, a r frequency-dependence of lung compliance was expected in cats than in adult humans. sumption was corroborated by our observation that lung compliance was reduced ean value of 25 per cent in the control situation when ventilation frequency was rised 5 to 50 per min. As far as we know frequency dependence of lung compliance in cats ver been studied before.

lowing collagen infusion the most striking reduction in lung compliance occurred er static conditions and the normal" breathing rate (24/min). This is in accordance the findings of Grimby *et al.* in man (1968). The finding of increased frequency

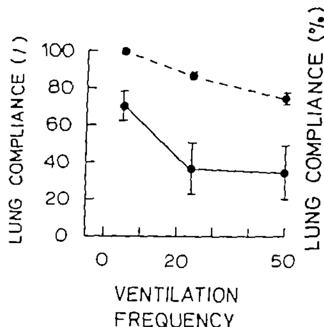


Fig. 3

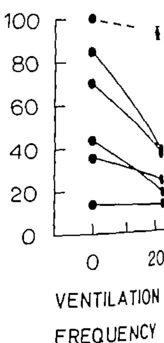


Fig. 4

Fig. 3 The co tinuous curve demonstrates the frequency-dependence of lung compliance after of collagen (10 infusions in 4 cats). The hatched curve demonstrates the control situation, before collagen infusion in the same animals. I this group lung compliance measured under semi-static (f = 5) was reduced by collagen infusions.

Fig. 4 Static and dynamic lung compliance (f = 24) measured before and after collagen infusion. The curve demonstrates the control situation (mean ± S D n = 5). The co tinuous curves change in lung compliance in each of the 5 animals. The more static compliance is reduced, the difference between values btained under static and under dynamic (f = 24) conditions.

Discussion

Previous experiments have shown that i.v. infusions of collagen set off a series of ev blood platelets aggregate and become trapped in the pulmonary vascular bed (Bø and Hog stad 1972) platelets will then during this aggregation release bioactive substances (Rådeg 1971 Vaage, Bø and Hognestad 1974) which cause constriction of pulmonary vessels airways Special attention has been paid to the role of prostaglandins in these ev (Rådegran 1971 Vaage and Piper 1975 Vaage 1976 c). Substantial experimental evide indicates that collagen has no smooth muscle action *per se* but acts only through its pla aggregating ability (Vaage, Bø and Hognestad 1974 Kobayashi *et al* 1974 Vaage 197

The aim of the present investigation was to gain more information about the air changes following induced intravascular aggregation. The expts. revealed that dyna lung compliance became highly frequency-dependent, a finding which suggests redu caliber of small airways.

Experiments on dogs have shown that resistance of airways smaller than 2 mm in diameter is a small component (*ca* 20%) of total pulmonary resistance (Macklem and M

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dependent behaviour of the cat lungs might theoretically be due to increased resistance in central airways. Woolcock *et al* (1969) used frequency dependence of compliance as a measurement of small airway obstruction only in the cases where lung distensibility and airway resistance were normal It is possible, however to differentiate between central and peripheral airway constriction after induced intravascular platelet aggregation in the presently used experimental model In previous expts. vagotomy and atropinization abolished approximately 50 per cent of the increase in non-elastic pulmonary resistance seen after collagen infusions (Vaage 1976 a) Such blocking did not, however influence the concurrent decrease in dyn C_L (Vaage 1976 a) Our interpretation is that central airway obstruction is not, or only to a very minor extent, a determinant of dyn C_L after induced intravascular platelet aggregation in cats. Indeed, due to the relatively greater contribution of peripheral airways to total airway resistance in cat lungs, the increase in non-elastic airway resistance remaining after vagotomy in these earlier experiments (Vaage 1976 a) might well be explained solely by increased flow resistance in the periphery Following this line of argument we conclude that the frequency-dependence of lung compliance observed after collagen infusions is most likely caused by constriction of small peripheral airways. These findings and conclusions are in agreement with studies on dogs on the effects of pulmonary emboli by blood clots (Hirose *et al* 1973) and with barium sulphate (Nadel, Colebatch and Olsen Pride 1970).

The combination of unchanged stat C_L and reduced dyn C_L (Fig. 2) indicates small airway *constriction without* any reduction in lung volume *i.e* no airway closure. When C_L is also *reduced while the fall* in dyn C_L is out of proportion to this reduction (Fig. 3 and 4), both *small airway closure* and narrowing must be present. Of particular interest are the ones with a major decrease in stat C_L (Fig. 4) Here frequency-dependence of compliance is almost eliminated A major part of the terminal airways must be closed in these lungs but mainly the central and medium-sized airways with their relatively small differences in time constants, patent and ventilated.

According to Mead (1969) and Macklem (1971) frequency-dependent behaviour of lungs is caused by 1) Asynchrony between conducting airways and the parenchyma (*i e* the gas exchange units) due to phase differences between these two compartments, and 2) Asynchrony between airspaces in parallel due to different time constants in different lung compartments as initially claimed by Otis *et al* (1956). Both these factors must be at play following intravascular platelet aggregation. Constriction of peripheral airways will slow down the gas flow in these lung units. Such constriction will not be evenly distributed since platelet aggregates are not located to all vascular areas in the lungs (Bo and Hognestad 1972). Therefore the release of constrictor substances will be uneven, leading to a more or less random pattern of constricted units

The above functional changes in peripheral airways will alter intrapulmonary gas distribution. With increasing inequality of time constants the end result will be disturbances in the ventilation-perfusion ratio and impaired gas exchange with arterial hypoxemia, such as seen in the various lung syndromes involving intravascular platelet aggregation.

Jarle Vaage has been a research fellow of the Norwegian Research Council for Science and the Humanities and is at present the recipient of a Research Fellowship from the University of Oslo. This work has also been supported by a grant from the Norwegian Council on Cardiovascular Diseases

Acta physiol. scand. 1977 100. 231–235

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nhibition of Gastric and Intestinal Motor Activity in Dogs by (Gln⁴) Neurotensin

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Received 23 December 1976

Abstract

ON, S., S Rosell, U Hjelmquist D Chang and K. Folkers. *Inhibition of tric and intestinal motor activity in dogs by (Gln⁴) neurotensin.* Acta physiol. scand. 7 *100* 231 235

n of (Gln⁴)-neurotensin as studied on the spontaneous motor activity in isolated canine fundic, d intestinal pouches All pouches had been prepared more than 6 months prior to the experiments. ous motor activity was recorded for at least 1 h before the (Gln⁴)-neurotensin was infused i. for i doses ranging between 6 3 and 100 ng kg^{-1} min^{-1} In the vagally denervated fundic pouches rotensin inhibited motor activity in doses bove 25 ng kg^{-1} min^{-1} The agally innervated ouches were more sensitiv than the vagally denervated fundic pouches to the action of (Gln⁴)-sin Thus motor inhibition as induced by doses as low as 6.3 g kg^{-1} min^{-1} The effect of eurotensin on motor activity in intestinal pouches was inconsistent Inhibition was seen in 1 expts. The present results show that the gastric motor activity is the most sensitive function)-neurotensin so far studied

ly w reported that the tridecapeptides neurotensin and (Gln⁴)-neurotensin produced ir effects in the small intestine at lower i. infusion rates than those necessary to uch effects in other vascular beds (Rosell *et al.* 1976). Moreover in conscious dogs eptides inhibit gastric acid secretion elicited by pentagastrin (Andersson *et al* 1976). findings may indicate that the gastrointestinal canal may be a target organ for tensin. Neurotensin was isolated from bovine hypothalamus, structurally elucidated nthesized by Carraway and Leeman (1976). Recently Folkers *et al* (1976) suggested Gln⁴)-neurotensin may be the naturally occurring peptide, rather than neurotensin. tensin may have been formed as a result of the extraction procedure. So far biological show that the two peptides have the same biological activity both *in vitro* (Rökaeus 1977) and *in vivo* In order to elucidat further the action of (Gln⁴)-neurotensin on astrointestinal tract, w have now studied its action on the spontaneous motor activity solated canine fundic, antral and intestinal pouches.

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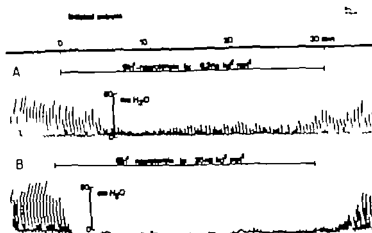
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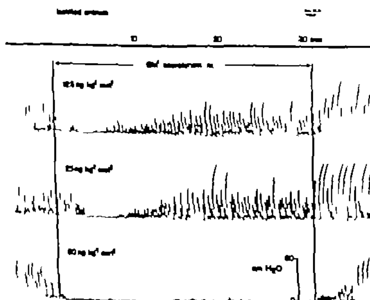
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Figs 1 & 2 B. Inhibitory effect of two doses (A and B) of (Gln⁴)-neurotensin on antral motility in one and the same dog

In contrast to the consistent inhibition of motor activity recorded from the gastric pouches, the motor activity of the intestinal pouches seemed more or less unaffected by the peptide at doses between 25 and 100 ng kg⁻¹ min⁻¹ (higher doses have not been tried). In only ... expts. did inhibition occur in the jejunal loop after 50 ng kg⁻¹ min⁻¹.
In no instance did the animals show any abnormal behaviour nor were any other adverse effects detected.



Inhibitory effect of three different doses of (Gln⁴)-neurotensin on antral motility in one and the same

Material and Methods

Motility studies were performed on 9 dogs (weight between 13 and 24 kg) which were provided with types of isolated gastrointestinal pouches. 5 dogs had Heidenhain pouches, 3 were equipped with innervated antral pouches and 1 dog had two isolated intestinal pouches, one duodenal and one jejunal (each about 15 cm). All pouches had been prepared more than 6 months prior to the expts In all, 26 expts were performed

Pressure changes in the pouches were measured using a Statham pressure transducer (P23 AC) connected to a Grass polygraph When measuring pressure activity in the Heidenhain pouches, 30–100 ml of a solution (pH 7) was introduced into the pouches and the Heidenhain pouch cannula was connected to the transducer by plastic tubing. Pressure changes in the antral pouches were recorded by means of a thin-walled rubber balloon connected to plastic tubing and containing between 10 and 30 ml of Each of the intestinal pouches had a modified Heidenhain pouch cannula and motor activity was recorded as in the Heidenhain pouches, but the volume of buffer solution introduced was only 10–20 ml.

The dogs were fasted fo 18–20 h prior to the tests. Spontaneous motor activity was recorded at least 1 h before the (Gln⁴)-neurotensin was administered. During the control period, a slow infusion (about 20 ml/h) of saline was given. (Gln)-neurotensin, in doses ranging between 6.3 and 100 ng kg⁻¹ min was then infused during 30–60 min periods. When repeated doses of (Gln⁴)-neurotensin were administered in the same expt., control periods of 30 min were allowed between the different doses.

Results

The Heidenhain pouches exhibited spontaneous motor activity with a mean frequency of 4 contractions per minute (range 3–5 per minute) Upon infusion of (Gln⁴)-neurotensin motor activity was inhibited (Fig. 1) The sensitivity of the different pouches to the inhibitory action of (Gln⁴)-neurotensin varied slightly but in all instances 100 ng kg⁻¹ min produced a clearcut reduction of motility and in some expts. inhibition was seen already at an infusion rate of 25 ng kg⁻ min

The antral pouches contracted with a frequency of about 3 contractions per min. Compared to the Heidenhain pouches the innervated antral pouches responded to lower infusion rates of (Gln)-neurotensin—in 1 expt to a dose of the peptide as low as 6.3 ng kg ×min (Fig. 2 A) 25 ng kg⁻ min produced complete inhibition of motor activity in the same dog (Fig. 2 B) The latent period from the start of the (Gln⁴)-neurotensin infusion to the occurrence of inhibition varied between 2 and 6 min. In many expts. there was an initial inhibition of pressure activity which then slowly returned to the control level despite continuous infusion of (Gln)-neurotensin. The result is illustrated in Fig. 3 which shows the effect of three consecutive doses of (Gln⁴)-neurotensin over 30 min intervals.

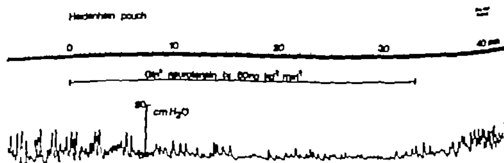


Fig. 1 Inhibitory effect of (Gln⁴)-neurotensin on Heidenhain pouch motor activity

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Discussion

The present study shows that (Gln[4])-neurotensin produces inhibition of gastric motility at very low concentrations The threshold dose for inhibition of antral motility was 5-10 ng kg^{-1} × min Previous expts. (Andersson *et al* 1976) have shown that gastric acid secretion is inhibited by neurotensin. A dose of 50 ng kg[-1] min neurotensin almost completely inhibited the acid response to pentagastrin in Pavlov pouch dogs. A similar degree of inhibition of gastric acid secretion was produced by (Gln[4])-neurotensin (unpublished). Furthermore secretory responses to a test meal and to insulin hypoglycemia can be inhibited by both peptides in Pavlov pouch dogs (unpublished) All these actions occur at lower levels than those which cause significant blood pressure changes or increase the blood glucose concentration (Rosell *et al* 1976) (Gln)-neurotensin (20 ng kg^{-1} ... has both vasodilator and vasoconstrictor actions, particularly in the splanchnic vascular bed (Rosell *et al* 1976) Carraway Kitabgi and Leeman (1976) have reported that the bovine small intestine contains 25–30 pmol/g wet weight of immunoreactive neurotensin. Taken together these observations indicate that the gastrointestinal canal may be a target organ for neurotensin or (Gln)-neurotensin

The different parts of the gastrointestinal tract varied in their sensitivity to (Gln[4])-neurotensin as far as motor activity is concerned Antral motility could be depressed by 6.3 ng × kg^{-} min but at least 25 ng kg^{-} min was required in the Heidenhain pouches. The motility in the isolated duodenal and intestinal pouches was even less responsive to (Gln[4])-neurotensin. Moreover in actute expts. on anesthetized dogs which were given doses of (Gln[4])-neurotensin that completely inhibited the pressure activity in the stomach no change in peristaltic activity of the small intestine could be seen by gross visual inspection (unpublished) The reason for these differences in the response to (Gln[4])-neurotensin in the gastrointestinal tract is not known at present One factor of importance may be innervation For example for inhibition of motor activity the vagally-denervated Heidenhain pouch required appreciably higher doses than the vagally innervated antral pouch. Furthermore, in order to achieve the same degree of inhibition of response to pentagastrin as that found in Pavlov pouch dogs, the Heidenhain pouch dogs required at least twice the dose of (Gln)-neurotensin (to be published) Further expts. are needed to show whether or not there is any synergism between vagal impulses and the inhibitory action of (Gln[4])-neurotensin on the oxyntic glands.

This study was supported by the Swedish Medical Research Council (no. 88), S ... Läkaresällskapet and the Robert A. Welch Foundation.

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Discussion

The present study shows that (Gln)-neurotensin produces inhibition of gastric [illegible] very low concentrations. The threshold dose for inhibition of antral motility was [illegible] $kg^{-1} \times min^{-1}$. Previous expts. (Andersson *et al* 1976) have shown that gastric acid [illegible] is inhibited by neurotensin. A dose of 50 ng kg × min neurotensin almost [illegible] inhibited the acid response to pentagastrin in Pavlov pouch dogs. A similar [illegible] inhibition of gastric acid secretion was produced by (Gln[4])-neurotensin [illegible] Furthermore secretory responses to a test meal and to insulin hypoglycemia [illegible] inhibited by both peptides in Pavlov pouch dogs (unpublished). All these actions [illegible] lower levels than those which cause significant blood pressure changes or [illegible] blood glucose concentration (Rosell *et al* 1976). (Gln)-neurotensin (20 ng kg [illegible]) has both vasodilator and vasoconstrictor actions, particularly in the splanchnic [illegible] bed (Rosell *et al* 1976). Carraway, Kitabgi and Leeman (1976) have reported that [illegible] vine small intestine contains 25–30 pmol/g wet weight of immunoreactive neuro[illegible] Taken together these observations indicate that the gastrointestinal canal may be [illegible] organ for neurotensin or (Gln[4])-neurotensin.

The different parts of the gastrointestinal tract varied in their sensitivity to (Gln[4])-neurotensin as far as motor activity is concerned. Antral motility could be depressed [illegible] 6.3 $ng \times kg^{-1} \times min^{-1}$ but at least 25 $ng \times kg^{-1} \times min^{-1}$ was required in the Heidenhain pouches. The motility in the isolated duodenal and intestinal pouches was even less responsive to (Gln)-neurotensin. Moreover, in acute expts. on anesthetized dogs which were [illegible] doses of (Gln[4])-neurotensin that completely inhibited the pressure activity in the stomach [illegible] change in peristaltic activity of the small intestine could be seen by gross visual [illegible] (unpublished). The reason for these differences in the response to (Gln[4])-neurotensin [illegible] the gastrointestinal tract is not known at present. One factor of importance may be [illegible] innervation. For example, for inhibition of motor activity the vagally-denervated Heidenhain pouch required appreciably higher doses than the vagally innervated antral [illegible] Furthermore, in order to achieve the same degree of inhibition of response to pentagastrin as that found in Pavlov pouch dogs, the Heidenhain pouch dogs required at least [illegible] dose of (Gln)-neurotensin (to be published). Further expts. are needed to show whether or not there is any synergism between vagal impulses and the inhibitory action of (Gln[4])-neurotensin on the oxyntic glands.

This study was supported by the Swedish Medical Research Council (no. [illegible]), Svensk Läkaresällskapet and the Robert A. Welch Foundation.

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Acta physiol. scand. 1977 100 236–245
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Blood Volume and Extravascular Water Content in the Rat Lung during Acute Alveolar Hypoxia

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Received 3 January 1977

Abstract

AARSETH, P and J KARLSEN. *Blood volume and extravascular water content in the rat lung during acute alveolar hypoxia* Acta physiol. scand. 1977 *100* 236–245

Values for pulmonary blood volume and extravascular lung water (estimated as wet weight of lung ... were arrived at in intact, anesthetized rats by labelling of blood constituents with isotopes and freezing the whole animals in liquid nitrogen. On ventilating the animals with 10 % O_2 in N_2/N_2O, a reduction in lung blood content could be demonstrated. The degree of reduction depended on the type of anesthesia and ventilation used. In some animals the volume reduction was so marked that both venous and capillary blood compartments have most probably been involved. The water content of the tissue was also rapidly and markedly reduced during hypoxia Increased plasma osmolarity in mixed blood could partly be responsible for this tissue water reduction.

Alveolar hypoxia is known to increase pulmonary vascular resistance in the isolated ... as well as in the intact organ (Von Euler and Liljestrand 1946, Duke 1957). Many investigations have been concerned with the vascular localization of the hypoxic response (for ... see Fishman 1976) As alveolar hypoxia is a more potent stimulus than systemic hypoxia, a postcapillary localization has been looked for and many investigators ... demonstrated an increased pulmonary venous resistance during alveolar hypoxia (F... Linden and Snow 1970 Hyman and Kadowitz 1975) Others have, however fo... effect on this segment of the lung vasculature (Doyle, Wilson and Warren 1952, Lloyd ... As P_{O_2} in the smaller precapillary lung vessels is dominated by alveolar P_{O_2}, these ... might well be responsible for the sensitivity to alveolar hypoxia With an histo... technique on rapidly frozen lungs, Kato and Staub (1966) have convincingly demon... that the small precapillary arteries constrict during alveolar hypoxia. According ... calculations, this constriction in the small arteries could fully explain the increased re... seen during alveolar hypoxia.

An outstanding feature of longlasting and marked alveolar hypoxia in intact anim... man, is pulmonary edema. Although increased precapillary resistance would seem ... to cause pulmonary edema, Severinghaus (1971) has presented evidence for a trans... water leakage taking place during alveolar hypoxia.

[illegible]re information about the vessel segments reacting to alveolar hypoxia could be gained [illegible] knew how the lung blood volume changed during such stimulation. We have measured [illegible] blood volume in intact rats given an hypoxic gas mixture. Tissue water content is [illegible]mined by the trans-capillary hydrostatic and colloid-osmotic forces, as well as by lymph [illegible] (Staub 1974). Acute changes in lung tissue weight (blood excluded) during hypoxia [illegible]ld therefore reflect changes in hydrostatic capillary pressure. Changes in the tissue water [illegible]nt could thus give another clue to the localization of the site of the vessels reactive [illegible]e hypoxic response.

[illegible]other reason for carrying out the present experiments follows from the fact that the [illegible] is an important blood depot, containing a large and changeable fraction of the total [illegible] d[illegible] olume. It would be of considerable interest therefore to know to what extent pulmo[illegible] blood volume is altered during hypoxia.

[illegible] preliminary report of the results has been given elsewhere (Karlsen and Aarseth 1976 a).

Methods

[illegible] Wistar rats weighing 205–215 g were used for all expts. The animals had free access to [illegible]ater and dry [illegible] food.

[illegible] animals were lightly anesthetized with ether and tracheotomized. In most of the expts. the rats were [illegible] breathing spontaneously. The tracheostoma was then connected to [illegible] wide tube generously flushed with the [illegible] breathing gas, [illegible]hich was initially about 20 [illegible] O_2 in N_2O. The composition and the volume flow of the gas [illegible] adjusted by separate gas flow meters, the P_{O_2} being controlled with [illegible] P_{O_2}-electrode (Radiometer). In [illegible] expts., the animals [illegible]are curarized (Alloferin[R] 0.1 ml/kg b.wt.) and ventilated [illegible]ith [illegible] Harvard ventila[illegible] pump. The frequency was 60–80 strokes per min and the inflation pressure 10 cm H_2O.

[illegible]atheters were placed in [illegible] femoral vein for injections and in [illegible] femoral artery for arterial blood sampling [illegible] pressure recording. Arterial pressure was measured [illegible]ith the catheter connected to [illegible] Statham transducer [illegible] Gb) and recorded on a Grass polygraph. Heart rate was counted from the pressure tracing.

[illegible]lmonary blood volume and weight of lung tissue proper were calculated using [illegible] method previously [illegible]ribed (Aarseth 1970). A known amount of [51]Cr-labelled rat erythrocytes and of [125]I-human plasma [illegible] was given [illegible] 8 min later [illegible] small blood sample (about 0.2 ml) was withdrawn, and the rat immersed [illegible] liquid nitrogen. The animal was dissected while still frozen and the lungs removed and weighed. Lung [illegible] volume was calculated from the isotope contents of the lungs and of the blood sample. The lung [illegible] weight was arrived at by subtracting the estimated weight of lung blood from the total weight of the [illegible]s. Rapid changes of lung tissue weight were taken as changes in extravascular lung water content.

[illegible]perimental groups. The effects of hypoxia on lung blood volume and tissue w[illegible]ter were arrived at [illegible]paring the values from control animals with those from animals given hypoxia. This was done in 3 [illegible] of animals. The groups differed as regards anesthesia and type of ventilation and were characterized [illegible] follows:

Group I N_2O anesthesia only.
Group II N_2O anesthesia with additional curarization and positive pressure ventilation.
Group III N_2O anesthesia with additional pentobarbitone-anesthesia (Nembutal[R] 30 mg/kg b.wt. given [illegible] p[illegible] or [illegible]).

In all expts., the administration of ether was stopped as soon as ventilation with the N_2O containing gas [illegible] started. One group of animals was maintained on N_2O-anesthesia. Additional anesthesia (pento[illegible]rbitone) or Alloferine was given to the animals in the two other groups. During [illegible] 30 min period blood [illegible] was then allowed to stabilize. Animals with arterial P_{O_2} below 70 mmHg at the end of this period [illegible] excluded. The rats [illegible]ere then given isotopes and the control animals were immersed in liquid nitrogen [illegible] min later. The test animals were ventilated with an hypoxic gas mixture (80 % N_2O, 10 % N_2, 10 [illegible] O_2) [illegible] 10 min before they were frozen.

There were only small differences between the groups as regards body weight and total blood volume [illegible] (Table I).

TABLE I Effect of alveolar hypoxia on 3 groups of rats. P_{FA} = femoral arterial pressure. PBV pulmonary blood volume. ETW = extravascular lung tissue weight. Median values and range.

Group	Anesthesia	n	Body weight (g)	Total blood volume (ml)	Arterial P_{O_2} (mmHg)	P_{FA} (mmHg)	Heart rate (Hz (S^{-1}))	PBV (ml)	ETW (g)
I	N_2O 80%	6	250 240–270	14.5 13 4–16.8	91 70–102	145 130–160	8.0 7.2–9.2	1.30 1.21 1.43	[illegible]
		5[b]	250 250–265	14 0 12.7–17 6	41 35–42	120 80–140	8.4 7.2–9.0	1 12 0.77–1 40	[illegible]
II	N O 80%	12[a]	250 230–270	14 0 11 1 16.7	10. 81 127	148 130–160	7.8 6 8–8 9	2 31 1 19–3 35	[illegible]
	Alloferine, (+ positive pressure ventilation)	8[b]	252 235–265	15 6 14.6–19.9	29 26–32	133 110–145	8.3 7 4–9 1	1.01 0.81 1.32	[illegible]
III	N O 80%	17[a]	224 210–285	14 6 11.8–17.3	93 77 118	130 75–150	7.8 6.4–9.0	1.88 1 49–2.94	[illegible]
	Nembutal 30 mg/kg	13[b]	234 205–270	14 9 12.0–16.3	30 26–33	70 40–95	8 4 7.2 9.0	1 40 1.01 1 61	[illegible]

Animals in normo ic condition
[b] Animals during hypoxia (after 10 min ventilation with 10% O_2 in N_2O and N_2).

Pulmonary arterial pressure was recorded in some preliminary expts. on animals given the same t as those in group II and III For cannulation of the pulmonary artery in the intact rat, a technique by Herget and Palecek (1972) was used A steel cannula was inserted through the right extern vein and advanced into the right atrium. Through this, and guided by pressure tracings, a thin catheter was further advanced into the right ventricle and then into the pulmonary artery Pu rterial pressure was recorded during a period with basic conditions, and then for 10 min th hypoxia.

Plasma osmolarity was measured in mixed venous blood from 14 rats. They were given the same t as described for groups II and III I addition a catheter was nserted through the jugular vein and int the right ventricle. When stable situation was achieved 1 ml of blood was withdrawn f catheter and replaced by nother ml of blood. The test animals (5 in group II and 6 in group III) v ventilated with 10% O_2 in N O/N for 10 min. A second blood sample was then withdrawn. In some a third sample was taken after another 20 min with normoxia. In 3 control animals no hypoxia troduced between the samplings.

The blood samples were centrif ged, and plasma osmolarity was measured by freezepoint deter using n Advanced Digimatic Osmometer (Advanced Instruments).

Statistical method. A Wilcoxon two-sample test for comparison of results from different groups of was employed.

Results

There were 3 groups of animals in which the effect of hypoxia was tested In each about half the animals served as controls and were not exposed to hypoxia.

The arterial P_{O_2} in the control animals ranged from 70 to 127 mmHg, median val the 3 groups being 91 102 and 93 mmHg (Table I) The rats given pentobarbitone III) had lower control blood pressure than rats in groups I and II (p = 0 0001) no difference between control animals from the different

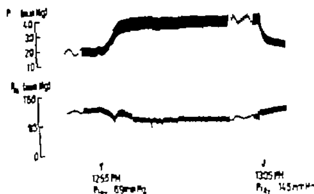


Fig. 1. Effect of hypoxia on pulmonary arterial pressure (upper tracing) and femoral arterial pressure (lower tracing) in cat given N_2O anesthesia, Alloferine and positive pressure ventilation. Catheters were placed in the femoral artery and through jugular vein into the pulmonary artery. Between the arrows O_2-tension of the ventilating gas (P_{IO_2}) was reduced to 69 mmHg.

When the animals were exposed to hypoxia by ventilating with a gas mixture containing 8% O_2, their arterial P_{O_2} was reduced to median values between 29 and 41 mmHg. During this exposure systemic arterial pressure was also reduced ($p < 0.016$). In the group given pentobarbitone, this reduction was very marked (Table I). Heart rate seemed to be increased in all groups during hypoxia, but the increases were not statistically significant.

The effect of hypoxia on pulmonary arterial pressure was evaluated in animals of group II given N_2O, Alloferine and positive pressure ventilation. As shown in Fig. 1 pulmonary arterial pressure increased rapidly during hypoxia, and this increase was maintained for a 10 min period. The maximum pressure increase in 4 expts. was 97% (median value, range 3–106%).

In 2 other expts. the pulmonary arterial pressure was measured in Nembutal-anesthetized animals during hypoxia. Also in these animals a hypoxic pressor response was evoked, but the response was apparently more moderate (25 and 87% increase in pressure, respectively).

The pulmonary blood volume in the control situation varied to a marked degree from one group of animals to another. In Table I the median values are given in absolute terms, while in Fig. 2 pulmonary blood volume is given as per cent of total blood volume for all expts. The animals in group II, ventilated by positive pressure, had more than 15% of their blood in the lung vessels (median value), *while in group I the corresponding value was* below 9% ($p = 0.002$). The pulmonary blood volume in group III animals (anesthetized with pentobarbitone) had an intermediate value.

The effect of hypoxia on pulmonary blood volume was also quite different in the 3 groups of animals (Table I, Fig. 2). In group I where the control value was low, there was only a small and insignificant further volume reduction during hypoxia ($p = 0.12$). In group II with its initial large value, the pulmonary blood volume was markedly reduced (56%). During this marked hypoxic reduction the pulmonary blood volume became as small as (or even smaller ($p = 0.085$)) than the ones found in group I during hypoxia.

TABLE I Effect of alveolar hypoxia on 3 groups of rats. P_{PA} = femoral arterial pressure. PBV = pul blood volume. ETW = extravascular lung tissue weight. Median values and range.

Group	Anesthesia	n	Body weight (g)	Total blood volume (ml)	Arterial P_{O_2} (mmHg)	P_{PA} (mmHg)	Heart rate (Hz (S^{-1}))	PBV (ml)	ET (g)
I	N_2O 80%	6	250 240–270	14.5 13.4–16.8	91 70–102	145 130–160	8.0 7.2–9.2	1.30 1.21–1.43	[illegible]
		5[b]	250 250–265	14.0 1_.7–17.6	41 35–42	120 80–140	8.4 7._–9.0	1.12 0.77–1.48	[illegible]
II	N_2O 80%	12[a]	250 230–270	14.0 11.1–16.7	102 81–127	148 130–180	7.8 6.8–8.9	2.31 1.19–3.35	[illegible]
	Alloferine, (+ positive pressure ventilation)	8[b]	_52 235–265	15.6 14.6–19.9	29 26–32	133 110–145	8.3 7.4–9.1	1.01 0.81–1.32	[illegible]
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	Nembutal, 30 mg/kg	13[b]	234 205–270	14.9 12.0–16.3	30 26–33	70 40–95	8.4 7._–9.0	1.40 1.01–1.61	[illegible]

[a] Animals in normoxic condition.
[b] Animals during hypoxia (after 10 min ventilation with 10% O_2 in N_2O and N_2).

Pulmonary arterial pressure was recorded in some preliminary expts. on animals given the same as those in group II and III. For cannulation of the pulmonary artery in the intact rat, a technique descr by Herget and Palecek (1972) was used. A steel cannula was inserted through the right external jug vein and advanced i to the right atrium. Through this, and guided by pressure tracings, a thin fiber catheter was further advanced into the right ventricle and then into the pulmonary artery. Pulmo arterial pressure was recorded during period with basic conditions, nd then for 10 min th alveo hypoxia.

Plasma smolarity was measured in mixed enous blood from 14 rats. They were gi en the same treat as described for groups II and III. I add tion catheter was inserted through the jugular vein and adva into the right entricle. When stable situation was achieved 1 ml of blood w s withdra from t catheter and replaced by nother ml of blood. The test animals (5 i group II and 6 in group III) were ventilated with 10% O_2 in N_2O/N_2 for 10 min. A second blood sample was then withdrawn. In some a third sample was taken after another 20 min with normoxia. In 3 control animals no hypoxia wa troduced between the samplings.

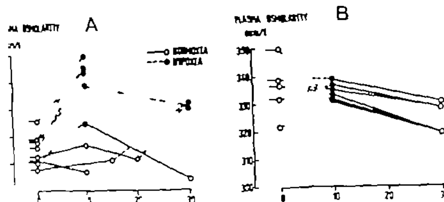
The blood samples were centrifuged, nd plasma osmolarity was measured by freezepoi t determin using an Advanced Digimatic Osmometer (Advanced Instruments).

Statistical method A Wilcoxon two-sample test for comparison of results from d fferent groups of a was employed.

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4 Plasma osmolarity in mixed venous blood from rats ventilated with 20 % O_2 (unbroken lines and open circles) or with 10 % O_2 (broken lines and filled circles). a. Animals anesthetized with 80 % N_2O and Alloferin. Positive pressure ventilation (group II). b. Animals anesthetized with pentobarbitone mg kg b wt. (group III)

Discussion

anesthetized rats, pulmonary arterial pressure increased markedly during ventilation th 10 % oxygen, and this pressure response could be maintained for 10 min with only a nor reduction in systemic arterial pressure.

Pulmonary blood volumes and lung tissue weights were estimated in 3 groups of rats posed to such a 10 min period of hypoxia, and the values were compared to those found normoxic control animals given the same treatment. There were marked differences tween the 3 control groups as both type of anesthesia and type of ventilation will influence lmonary blood volume and tissue water content (Aarseth 1972, Karlsen and Aarseth 77). There were also small differences as regards arterial P_{O_2} in the control animals. The essor response of hypoxia will, however not be present if P_{O_2} is kept above 55 mmHg large and Staub 1969). In the present expts., animal was discarded if arterial P_{O_2} was low 70 mmHg before the experimental period.

During the sustained period of hypoxia, the pulmonary blood volume decreased, although t to the same degree or to the same level in all groups. The animals receiving only N_2O ere lightly anesthetized, and the stress caused by the tracheostoma and the vascular cannulation per se would reduce pulmonary blood volume, conceivably via sympathetic nerv ivity (Aarseth 1972). In such animals hypoxia caused only small further reduction pulmonary blood volume down to a median value of 7.2 % of total blood volume. This actually close to the lowest capacitance seen for the lung vessel of rats (Aarseth 1972).

The animals which were curarized and ventilated by positive pressure had, on the other hand, a very large part of their blood in the lung vasculature. This was probably due, to large extent, to the increased alveolar pressure (Karlsen and Aarseth 1977). In such animals hypoxia was a very potent stimulus for capacitance reduction of the lung vessels (Fig. 2), which then expelled about 56 % of their blood content.

G ... proper anesthesia the spontaneously ventilating control animals also had a large pulmonary blood volume which was reduced during hypoxia (Fig. 2, group III). However

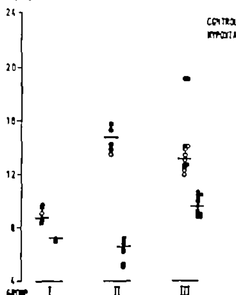


Fig. 2

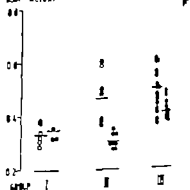


Fig. 3

Fig. 2. Effect of hypoxia on pulmonary blood volume which is given as percent of total blood vol
3 groups of rats. All animals were given 80% N_2O. In addition, the group II animals were curarized
ventilated with positive pressure, while group III animals were given Nembutal 30 mg/kg b.wt. Horiz
bars show median values.

Fig. 3. Effect of hypoxia on lung tissue weight in 3 groups of rats (see legend to Fig. 2). The weights, w
represent lung tissue without blood, are given as per cent of b.wt. Horizontal bars show median val

Also in the animals anesthetized with pentobarbitone (group III) there was a signifi reduction of pulmonary blood volume during hypoxia ($p < 0.01$). The reduction was, h ever, less marked than in the group II animals, and the minimal pulmonary blood co reached during hypoxia was larger in these animals than in those of groups I and II ane tized with only N_2O ($p < 0.01$).

The median values for weight of extravascular lung tissue are given in Table I. In F this parameter has been calculated as percent of total body weight and is shown fo expts. The median lung tissue weights in the control animals of the groups II and III about 0.50% of body weight, while in group I it was only 0.33% and thus signific smaller than in the other groups ($p < 0.001$). During hypoxia the lung tissue weight unchanged in group I. In group III there was some reduction and in group II there w marked reduction amounting to 370 mg or almost 1/3 of the wet lung weight ($p < 0.01$).

Plasma osmolarity of mixed venous blood was examined in animals from group II (anesthesia and Alloferin) and group III (pentobarbitone anesthesia). At least one pl sample was taken during normoxic conditions, after which hypoxia was induced. An plasma sample was then obtained after 10 min of hypoxia. In the animals of group III was no consistent change in plasma osmolarity during hypoxia (Fig. 4 b), whereas the an of group II showed an increase of 14–30 mosm/l (median increase 20 mosm/l) (Fig. Two or three consecutive plasma samples were taken and tested at 10 min intervals d normoxic condition in 3 animals of group II. There were hardly any changes in pl osmolarity from one test to another.

a using an analogue model, Piene (1976) calculated that there was a 35% reduction of the ·rial volume in the cat lung during hypoxia.

Even the most drastic reductions of arterial and capillary blood volumes can not, however ·y explain the reduction of lung blood content in the group II animals from 2.3 to 1.0 ml. ·e venous compartment must have been involved as well. Morgan *et al.* (1968) have actually ·asured the diameter of pulmonary veins and shown them to be reduced during hypoxia. ·rom these considerations it appears logical to conclude that hypoxia causes a widespread ·l marked constriction in both arterial and venous vessels in the rat lung.

·Changes in lung water content could give further quantitative information about the ·omotor response in the different segments. It is remarkable that within few minutes the ·tissue weight of the rat lung changes as markedly as in Fig. 3. Methodological errors ·t could explain this strange finding, have been looked for without success (Aarseth ·77). We therefore conclude that the changes are real and reflect a changed extravascular ·ter content. As the alveolo-capillary membrane constitutes a relatively large fraction of the ·g of small animals, marked transvascular water flux might occur in such lungs.

However with reasonable capillary filtration coefficient for the rat lung (Staub 1974) · transcapillary forces in the group II animals had to be altered by some 150 mmHg, · order to explain the actual tissue water reduction. Our usual concept of the Starling-·lance, where only colloid osmotic and hydrostatic pressures are involved, thus appears · be too simple in this case. In a recent series of publications Järhult and his coworkers ·rhult 1975) have demonstrated that other substances than plasma proteins, mainly glucose, ·ve important transvascular osmotic effects. Such a mechanism might operate also in the ·esent experiments as plasma osmolarity increased by about 20 mosm/l in the group II ·imals. It ·ppears likely that glucose is responsible for this osmolarity change (Järhult ·75). With a reflection coefficient of 0.2 for glucose, 20 mosm/l would represent an osmotic ·essure of about 58 mmHg. In situations where glucose is continuously released from the ·er it might maintain a marked osmotic water drag across the capillary membrane. Fur·ermore, it would efficiently remove also cellular water favouring water transport into the ·scular compartment. It has previously been demonstrated that when plasma osmolarity · increased by glucose-infusion, the lung water content will be markedly reduced (Piene · *al.* 1974). The lung water content could therefore be reduced, even if the hydrostatic ·essure was increased in some capillaries during hypoxia.

From these considerations it can be assumed that the release of glucose from glycogen ·tores may increase the safety factor against high altitude pulmonary edema. In this con·ection it is interesting to mention that this type of edema is often observed after heavy ·uscular exercise (Herbert *et al.* 1962). In this ·ituation the liver glycogen content might be ·markedly reduced.

A lung water content was reduced to a small extent also in animals with no blood osmo·arity change, other factors must be involved as well. Karlsen and Aarseth (1976 b) have ·roposed that there exists a correlation between pulmonary blood volume on the one hand, ·nd extravascular lung water content on the other. So far we have no definite explanation ·s to ·hat lies behind such a correlation, but other mechanisms than changes in the pre-·o post-capillary resistance ratio must be involved.

the pulmonary blood volume was not reduced to the same low level as in group I rat animals. The ventilation of 10% O was thus not as effective a stimulus for pulmonary capacitance reduction in Nembutal-anesthetized animals as in N_2O-anesthetized ra although arterial P_{O_2} was reduced to the same level (Table I). The capacitance reduc effect of hypoxia might be attenuated by pentobarbitone, as has been described for t pulmonary pressor effect of alveolar hypoxia (Susmano, Passovoy and Carleton 19 It is also possible that during pentobarbitone anesthesia pulmonary blood is conta within vessels less influenced by alveolar hypoxia.

Given the proper basic conditions, alveolar hypoxia, with an arterial P_{O_2} of 30-40 mm will markedly reduce the pulmonary blood volume in the intact rat. This is in accordan with previous results obtained on cat lungs, using a biopsy-technique (Aarseth, Karle and Bø 1975) The same type of results was arrived at during hypoxia in fetal lamb by R *et al* (1975) Also Honig and Tenney (1957), using a teeterboard, found a reduction central blood volume in dogs ventilated with 12 % O Other investigators have, howev found no change in central blood volume during hypoxia in dog and man (Doyle *et a* 1952, Fritts *et al* 1960) Using the Stewart Hamilton method in dogs Stroud and Co (1954) and Nahas *et al* (1954) have even reported an increased central blood volume dur hypoxia. There may be species differences in the effect of hypoxia on lung blood volum

The marked reduction of the lung blood volume presently seen in rats during hypo calls for considerations about which section of the vasculature could be involved.

In the adult rat the lung capillary volume has been estimated to be about 1 1 ml (We 1967) If this holds also for the animals in the present expts., the capillary blood volum would make as much as 50% of the total lung blood volume. With such a partition no sin vessel compartment can be responsible for a 56% reduction of lung blood content. In ad tion to the capillaries either the arteries or the veins must be involved

A reduction in capillary blood volume can only be brought about by a reduction transmural capillary pressure. There is no reason to believe that extravascular press *i.e* alveolar pressure, should be changed during hypoxia As to the intravascular capill pressure, it depends on flow and postcapillary resistance. In the present expts. card output has not been measured However blood flow through the lung is increased dur hypoxia in the dog (Stroud *et al* 1954) and in the cat (Aarseth *et al* 1975). It has also bee shown that pulmonary venous resistance increases in this situation in the dog (Rivera *et a* 1958 Furnival *et al* 1970) and in the sheep (Hyman *et al* 1975) A reduced capillary volum can then only be achieved if there is increased pressure in some perfused capillaries, wh others are non-perfused and collapsed due to precapillary vasoconstriction.

The present findings thus give further evidence for a precapillary vasoconstriction taking place during alveolar hypoxia. However if this constriction is located only in the small pulmonary arteries, as suggested by Kato *et al* (1966) the remaining arterial tree would b distended by the increased arterial pressure. Actually with a pulmonary arterial com pliance similar to that in the dog (Shoukas 1975) and with the pressures prevailing in th present expts., the distension could amount to a volume increase of 1 ml in the rat. W the present findings there is no room for such an expansion, and active vasoconstriction throughout the arterial segment must have taken place From changes in vascular impedan

using an analogue model, Piene (1976) calculated that there was a 35% reduction of the
lal volume in the cat lung during hypoxia.
en the most drastic reductions of arterial and capillary blood volumes can not, however
r explain the reduction of lung blood content in the group II animals from 2.3 to 1.0 ml.
venous compartment must have been involved as well. Morgan *et al.* (1968) have actually
sured the diameter of pulmonary veins and shown them to be reduced during hypoxia.
rom these considerations it appears logical to conclude that hypoxia causes a widespread
marked constriction in both arterial and venous vessels in the rat lung.
hanges in lung water content could give further quantitative information about the
motor response in the different segments. It is remarkable that within few minutes the
tissue weight of the rat lung changes as markedly as in Fig. 3. Methodological errors
could explain this strange finding, have been looked for without success (Aarseth
7). We therefore conclude that the changes are real and reflect a changed extravascular
er content. As the alveolo-capillary membrane constitutes a relatively large fraction of the
g of small animals, marked transvascular water flux might occur in such lungs.
lowever with a reasonable capillary filtration coefficient for the rat lung (Staub 1974)
transcapillary forces in the group II animals had to be altered by some 150 mmHg,
order to explain the actual tissue water reduction. Our usual concept of the Starling-
ance, where only colloid osmotic and hydrostatic pressures are involved, thus appears
be too simple in this case. In a recent series of publications Järhult and his coworkers
rhult 1975) have demonstrated that other substances than plasma proteins, mainly glucose,
ve important transvascular osmotic effects. Such a mechanism might operate also in the
sent experiments, as plasma osmolarity increased by about 20 mosm/l in the group II
imals. It appears likely that glucose is responsible for this osmolarity change (Järhult
75). With reflection coefficient of 0.2 for glucose, 20 mosm/l would represent an osmotic
essure of about 58 mmHg. In situations where glucose is continuously released from the
er it might maintain a marked osmotic water drag across the capillary membrane. Fur
ermore, it would efficiently remove also cellular water favouring water transport into the
scular compartment. It has previously been demonstrated that when plasma osmolarity
increased by glucose-infusion, the lung water content will be markedly reduced (Piene
al. 1974). The lung water content could therefore be reduced, even if the hydrostatic
ressure was increased in some capillaries during hypoxia.

From these considerations it can be assumed that the release of glucose from glycogen
tores may increase the safety factor against high altitude pulmonary edema. In this con-
ection it is interesting to mention that this type of edema is often observed after heavy
uscular exercise (Herbert *et al.* 1962). In this situation the liver glycogen content might be
markedly reduced.

As lung ater content was reduced to a small extent also in animals with no blood osmo-
arity change, other factors must be involved as well. Karlsen and Aarseth (1976 b) have
proposed that there exists a correlation between pulmonary blood volume on the one hand,
and extravascular lung water content on the other. So far we have no definite explanation
as to what lies behind such correlation, but other mechanisms than changes in the pre
to post-capillary resistance ratio must be involved.

From the blood volume considerations it appears that a widespread constriction of the vessels takes place during alveolar hypoxia in intact rats. Usually only the resistance change during hypoxia has been studied This last change may be fully explained by constriction in some minor arterial segments. It is conceivable therefore that the classical hypoxic pressor response, located in smaller resistance vessels, and the widespread lung blood volume reduction upon hypoxia is not exactly one and the same response. The latter could well be mediated in a different and more complex way than the former In his recent review on the mediators of the pulmonary vascular response to hypoxia, Fishman (1976) was only concerned with the increased resistance. The local release of histamine may well be responsible for the pressure response (Hauge 1968), but is not likely to mediate the whole, widespread volume response described in the present paper It would be interesting to study the possible influence of sympathetic nerves on this volume reduction response. There is ample evidence that the sympathetic nerve system or α-receptors are not obligatory components for the hypoxic pressor response (Lloyd 1966, Thilenius, Candiolo and Beug 1967) but an attenuation of the response by α blockade has been reported (Porcelli and Bergofsky 1973) The smaller volume reducing effect of hypoxia in the pentobarbitone anesthetized animals of the present experiments is worth mentioning in this connection. One possible explanation here might be the sympathicolytic effect of pentobarbitone.

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...BLE 1. Physical characteristics of subjects with respect to age, ...eight, height and percent distribution of slow twitch fibres (...ST) in the vastus lateralis muscle.

...bject	Age (yrs)	Weight (kg)	Height (cm)	ST %
	28	77.2	183	60
	24	76.3	179	77
H	24	83.0	185	59
L	4	80.6	186	59

...eated maximal eccentric contractions of elbow flexors (Komi and Rusko 1974). This ...scle soreness may in some instances last for several days. Thus the recovery processes ...apparently delayed after high tension eccentric loading. This study was therefore designed ...investigate electrical and metabolic aspects of the quadriceps muscle group during and ...ew days following the fatigue loading of concentric and eccentric work.

Methods

...jects and experimental design

...hysical education students volunteered as subjects. Their physical characteristics are shown in Table 1. ...ch subject performed 2 types of work tests, concentric and eccentric. In both conditions the subjects ...erted 40 maximal contractions. Each contraction lasted for 3.5 ... and the intercontraction pause ...as 11.5 ... Thus the total ...ork time with pauses averaged 10 min. Fig. 1 depicts the experimental design showing the ...fferent parameters recorded or sampled during and ...few days following the loading period.

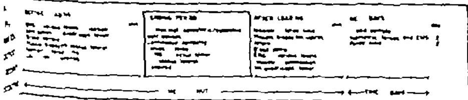
...ynamometer for fatigue loading

...electromechanical dynamometer (Komi 1973 b, Fig. 2) was used for loading the lower limb extensors ...d for recording the exerted forces during the contraction phase. The subject was seated on the dynamo-...ter, in ...hich the foot plate moved ...ith ... constant velocity ...way from and toward the subject, respectively ...concentric and eccentric work. In both cases the exerted forces were recorded with strain gauges during ...entire linear movement range, ...hich averaged 38 cm allowing intersubject variations due to differences ...in lower body segmental parameters. No plantar flexion took place during movement. Thus the work ...in both types of contractions ...as assumed to be performed primarily by the quadriceps muscle group and ...condarily by the gluteal muscles.

...Force-time curve in maximum isometric contraction of the both leg extension was registered before, ...mmediately after and on the second day after the fatigue loading. I... this test the knee angle was set at ...7° and the subject was instructed to exert the maximum force as fast as possible.

...lectromyographic measurements

...lectromyographic (EMG) activity was measured utilizing the surface electrode recording from both the ...ctus femoris and vastus lateralis muscles. The Beckman miniature size electrodes (contact diameter 4 mm)



...mental scheme for the measurement of the various parameters before, during and after the

Acta physiol. scand. 1977 100 246–254

From the Kinesiology Laboratory Department of Biology of Physical Activity
University of Jyväskylä, Finland

Changes in Motor Unit Activity and Metabolism in Human Skeletal Muscle during and after Repeated Eccentric and Concentric Contractions

By

PAAVO V KOMI and JUKKA T VIITASALO

Received 25 January 1977

Abstract

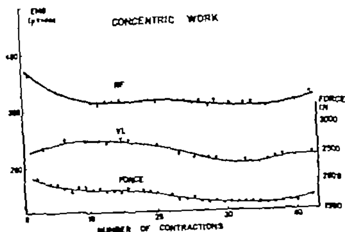
KOMI, P V and J T VIITASALO. *Changes in motor unit activity and metabolism in hu[man] skeletal muscle during and after repeated eccentric and concentric contractions.* A[cta] physiol. scand. 1977 *100* 246–254

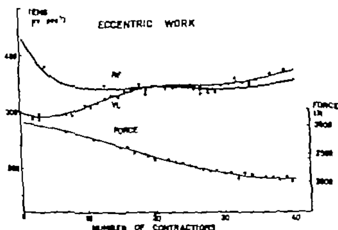
This study was designed to investigat electromyographic (EMG), muscle glycogen and blood la[ctate] changes in quadriceps muscle group during repeated 40 maximal eccentric and concentric contra[ctions] and to follow the recovery in EMG muscle glycogen and serum creatine kinase values during a 4[8 h] period following the wo k test. The subjects were normal males and the test order (eccentric or concen[tric]) was randomly selected. The results indicated first, that th EMG parameters (IEMG, AMUP), m[uscle] glycogen and blood lactate changed in similar manner during the both f tigue loads. Despite the [high] tension work no selective depletion of glycogen could be observed in the slow or fast twitch muscle f[ibres] i either type of work. The restoring of muscle glycogen occurred similar manner after the both [fatigue] loads, and no significant differences were present between eccentric and concentric works in the s[erum] creatine kinase levels f 2 days period. The eccentric wo k was associated with muscle soreness, [which] was strongest during the second day after the termination of the work test. The recovery of the E[MG] parameters were also delayed in eccentric fatigue. After concentric f tigue EMG-activity returned to [pre] values within 2 days.

Key words. Eccentric work, electromyography muscular fatigue, muscle soreness

Eccentric work is characterized by smaller expenditure of energy (*e.g* Asmussen 1953) lower motor unit activity (*e.g* Bigland and Lippold 1954) than concentric work perfor[med] with similar muscular tension and constant contraction velocity In both types of work activation of muscle increases linearly with the increase in force output, but the slope— IEMG/tension—is much lower in eccentric work (Komi 1973 a) Production of m[uscle] tension can be much higher in eccentric contraction and the difference in tension bet[ween] maximal eccentric and concentric work increases with the increase in contraction vel[ocity] (Asmussen *et al* 1965 Komi 1973)

Most probably due to the high production of tension the muscle becomes easily in early stages of eccentric training (Komi and Buskirk 1972) or after the fatigue loading





ntegrated electromyographic activity (IEMG) of the rectus femoris (RF) and vastus lateralis (VL) and the force during the course of 40 maximum contractions of concentric (upper) and eccentric work.

1180 N to 2080 N (Δ 34.6%) and from 1923 N to 1669 N (Δ −13.2%), respec he eccentric and concentric work.

IG hich was also analyzed for the mid-portion of the movement and in comparable e lengths of both conditions showed a fast reduction in the early work, but then relatively constant during the entire work period of the both conditions. IEMG y of the vastus lateralis muscle tended to decrease slightly during concentric f tigue, owed similar nonsignificant increase in eccentric fatigue (Fig. 3).

e relationship between IEMG and muscle tension in isometric knee extension showed, ver comparable changes in both muscles so that substantially more neural energy eeded for the production of a certain muscle tension after than before the fatigue ng. This kind of shift in the regression line to the left (Fig. 4) was greater in eccentric

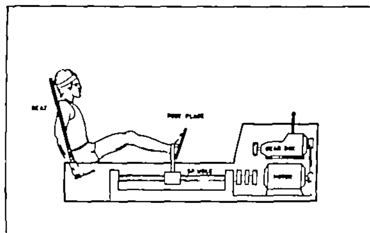


Fig. 2 The electro dynamometer for load lower limb extensors in c and eccentric work

were placed on the belly of the rectus femoris muscle. On the vastus lateralis muscle the electro placed on the position corresponding the point of biopsy sampling from the contralateral mu this electrode pair picked up the activity primarily from the middle portion of the muscle. Inter distance for both muscles was 3 cm. The EMG signals were amplified with Tektronix RM low le fiers by using the gai of 60 dB and the lower and upper cut off frequencies of 10 Hz and 10 00 spectively The mplified EMG and force signals were stored on magnetic tape (Philips analog-7) sequent off line analysis with HP 2116C computer This analysis gave the following EMG pa Integrated EMG (IEMG), Averaged Motor Unit Potential (AMUP), and the mean power (MPF) of the power spectrum density function (for details see Viitasalo and Komi 1975, Komi and 1976)

EMG was recorded continuously d ring the work loading, but also before the work and soon termination of the w k, nd on the second day after the test (see Fig. 1) I Isometric one leg knee (see Viitasalo and Komi 1975 for dynamometer construction and testing details) employi g the submaximal loads 150, 250, 400 550 and 700 Newtons. After the fatigue loading the 700 N load too heavy and thus could not be lways recorded.

Muscle biopsies The standardized technique of Bergström (1962) was used to obtain the s muscle tissue before and immediately after both eccentric and concentric loadings from the sam muscle. 2 samples were taken in each case. One of them was used for serial cryostat stainings fo ATPase (Padykula nd Herman 1955) and PAS (Pearse 1961), respectively f r identification o twitch (FT) and slow twitch (ST) f bres (Gollnick t al. 1972) and for qualitative bservation of content in the different f bres. The other sample was used for biochemical analysis of muscle content. This result was expressed as g glucosyl units/kg muscle dry weight.

Oxygen uptake was measured during the work load through collection of the expired air in the bags. O_2 and CO_2-contents were analyzed with the routine Scholander method

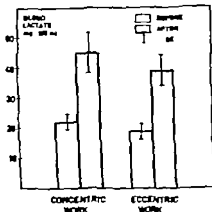
Lactate was analyzed from the venous blood sample taken before and 4-5 mi after the test. Th of *serum creatine kinase* (CK) was lso nalyzed for these time periods nd also followed for a after the test (see F g. 1) Biochemica-Boehri ger test packages were used in these nalyses.

Results

The presentation of the results is divided into 2 parts Firstly primary changes in t meters during the fatigue loading and secondly inspection of the attributes whi followed for a few days after the test

Primary changes during fatigue loading

Muscle tension as averaged for the mid portion of each contraction decreased subs more in eccentric than in concentric fatigue condition (Fig 3). The average decrea



6 Values for blood lactate before and after concentric and eccentric ork loadings

glycogen utilization from the two fibre types indicated a general loss of glycogen, but selective depletion could be observed in the ST or FT fibres.

ecovery from fatigue

uring the recovery period of 4 days most of the tests were repeated on the 2nd day and the alysis of the serum creatine kinase on each successive day. The results indicated that in uscle tension and EMG variables the recovery was complete after concentric work, but bstantially delayed after eccentric work (Fig. 4). In fact after eccentric work no return the before-test condition occurred either in force-time (Fig. 8) or IEMG/muscle tension riables. 2 days after concentric work most of the subjects were able to exert greater forces an before the work test. Fig. 9 demonstrates how the AMUP curves from the EMG alysis were only slightly different in concentric condition for the three test instances efore, after and 2nd day), but were substantially separated in eccentric work. Especially oticeable is the large AMUP-amplitude at 250 N load on the 2nd day.

In neither conditions did muscle glycogen content return to normal levels on the 2nd ay after the tests (Fig. 7). The serum CK value stayed at slightly elevated levels for few ays after the test, and was higher (n.s.) in eccentric than in concentric condition (Fig. 10).

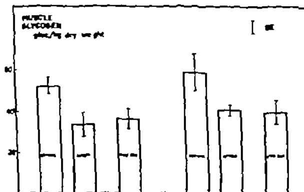
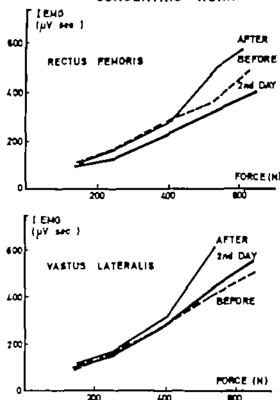


Fig. 7 Muscle glycogen levels before, after and two days after the work loadings



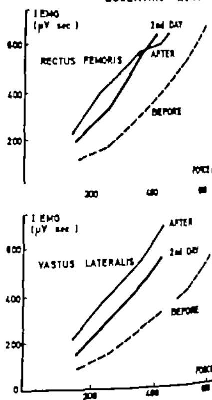


Fig. 4 Relationship between IEMG and isometric f rce for rectus femoris and vastus lateralis m before, immediately after and two days after the fatigue loading in concentric and eccentric ork.

than in concentric work. No significant changes could be seen in the power spectral p meters in isometric test conditions. AMUP however was increased and substantially in this test after eccentric work (Fig. 9)

The curves for oxygen consumption demonstrated similar patterns in both types of fa conditions (Fig. 5) Similarly blood lactate increased (Fig. 6) and muscle glycogen decre (Fig. 7) in the same manner for eccentric and concentric work. The histochemical an

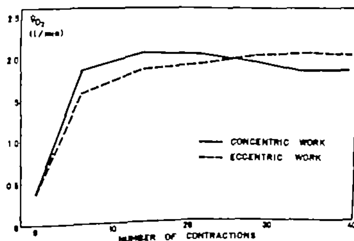
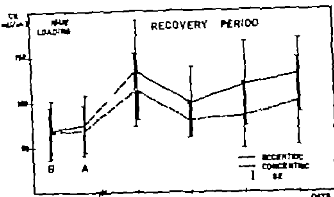


Fig. 5 Mean o ygen consu (VO_2) for all subjects duri course of f tigue loading eccentric and concentric wo



Serum creatine kinase levels (CK) before (B), after (A) and for few day following the ork

e two work conditions differed more pronouncedly as regards the parameters followed few days after the test session. The results of the force-time curve measurements . 8) and AMUP analysis of EMG records (Fig. 9) showed that the performance of the romuscular system was substantially weakened two days after the eccentric fatigue. he same time all the subjects complained of severe muscle soreness.

he decreased strength performance of human skeletal muscle has been described to be partly due to the lowered ATP concentration in the working muscles (Jorgensen and erich 1976). ATP concentration was not measured in the present study and thus annot directly be argued that it stayed at different levels after eccentric than concentric k. It is reasonable to assume, however that the ATP levels were not different, and that reasons for the delayed recovery after eccentric fatigue may be due to some other changes he muscle.

ur earlier reports (Komi and Rusko 1974 Komi *et al.* 1974) and that of Asmussen 56) emphasize the appearance of muscle soreness after eccentric work as compared to centric work. The symptoms of soreness have also been shown to accompany the reduc n of strength performance during early eccentric training (Komi and Buskirk 1972). soreness experienced by the present subjects is in accordance with these earlier observa- n. However it still seems unclear what the exact site and mechanism for the soreness is. ussen (1956) suggests that the soreness is caused by swelling of or in the connectiv e, which ill then be distended during contraction at right angles to the direction of .

oreness is probably also the cause for increased neural activation of the muscle at a given sion during the few days after the eccentric work (Fig. 4) It is natural to expect that more ral activation is needed for a sore muscle as compared to the healthy muscle. Fig. 9 the AMUP analysis is an indication of this.

The measurements of the activity of the serum CK enzymes showed no significant differ ces between the two work types. Also our earlier attempt to follow the possible ultra- ctural changes after the ...

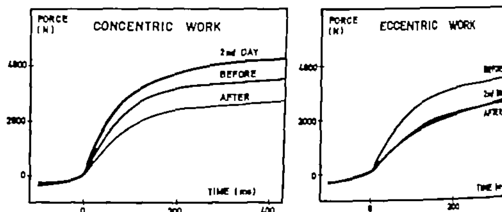


Fig. 8 Force-time curves before after and two days after the work loadings.

As expected from earlier work (Komi and Rusko 1974) the repeated eccentric loa contrast to concentric work caused severe muscle soreness, which was strongest on the and third day after the work test

Discussion

The fatigue loadings used in the present study were able to demonstrate the following differences in the behavior of the investigated muscles during work Firstly the eccentric fatigue loading with maximal repeated contractions caused a greater reduction in tension than the corresponding maximal concentric loading. This is in agreement with previous finding with elbow flexors (Komi and Rusko 1974) and leg extensors (Komi et 1974) Secondly from the measured EMG parameters, AMUP and IEMG as measured isometric conditions immediately before and after the work sessions, indicated that eccentric work was more "fatiguing" than the concentric work. EMG analysis from the contractions themselves did not however differentiate markedly the behavior of the investigated muscles between the two loading conditions. Similarly histochemical and biochemical analysis of muscle glycogen levels of blood lactate or the total oxygen consumption did not reveal any significant differences between concentric and eccentric work.

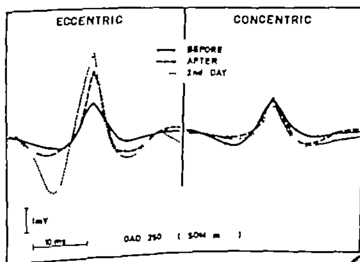


Fig. 9 A erage motor unit potentials (AMUP) recorded from rectus femoris muscle in maximal isometric contraction before, fter and two day after work loadings.

Acta physiol. scand. 1977. 100. 255-257

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Emotional" Bradycardia. A Telemetry Study on Incubating Willow Grouse (*Lagopus lagopus*)

By

GEIR GABRIELSEN, JOHN KANWISHER and JOHAN B. STEEN

gh heart rate in most species reflects the metabolic state of the animal, emotional may act as strong modifying agents. This has been experienced by arctic explorers ged by polar bears (Nansen 1898) and by TV-watchers during exciting shows. It noticed in animals under a variety of experimental conditions (Folkow and Neil Thus, in some animals, cortico-hypothalamic activity may strongly influence the vascular response pattern in certain situations. Inexperienced parachuters, for in-, may more than double their heart rate, without any increase in metabolism, prior mp (Ursin, Baade and Levine 1977). Opossums and deerkids, on the other hand, y pronounced cardiac retardation, much reduced breathing rate and temporary loss sciousness ("playing dead"-reaction) when exposed to danger (Folkow and Neil . In this context it is noteworthy that the cardiac response to danger in arctic explorers not involve loss of consciousness. This was well demonstrated when H. Johansen from pine position underneath a hungry bear exclaimed to his companion, F. Nansen: must look sharp if you want to be in time" (Nansen 1898).

this paper we describe pronounced bradycardia in incubating, wild, willow grouse tened by intruders. Domesticated individuals of the same species were also studied, but animals did not display any bradycardia response.

tudy was carried out in June 1976, the field work was done Kurløy, an island in Tromsø county ° N. The indoor work was done at the Wildlife Research Station, University of Tromsø.

e incubating willow grouse hens, localized by pointing dogs and caught on the nest by dip nets, were ped with telemetric equipment. This included an ECG preamplifier, modulator and crystalcontrolled mitter on 102.36 MHz. With batteries for 400 h operation and transmission range up to 200 m, the weight was 13 g. The transmitter was taped to grouse rucksack snugly strapped around the shoulders e bird. One gold-tipped wire from the ECG amplifier was positioned close to the heart apex by means length of stiff plastic tubing introduced through small incision behind the sternum. The other rode was placed under the skin overlying the breast muscle. The procedure lasted about 15 min. The would usually return to the nest within 20 min after release and take up her brooding chores, apparently urbed by the treatment.

he signals from the transmitter were picked up by directional Yagi antenna and commercial FM- and recorded on cassette tape for later analysis. Direct recording of ECG signals was made and -to-beat heart rate was later derived by means of an on-line micro-computer.

len 1 was at least two years old, hen 2 was one year old as shown by the pigmentation of their wing

reticulum or in the organization of the contractile proteins and z bands. Thus it ma concluded that the great mechanical stress during maximal eccentric work caus structural changes in the muscle leading to severe soreness, which is accompanied by rel neuromuscular performance. However the mechanisms involved are yet unclear

Supported in part by a grant (No 9285/79/72) from the Ministry of Education (Finland)

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xiet, although her heart rate always increased 20–50%. The cardiac activity of both increased when we knocked on the cage or made other unusual sounds.

...nwisher *et al* monitored the heart rate of a sea-gull which had its favourite vantage ...t on top of his flag pole in Woods Hole. Whenever he coughed or shouted, the gull ...oled its heart rate (from 200 to 400 BPM) without visual indications of excitement. It ...markable that while the wild grouse exhibits a similar cardiac response to mild (re-...e) provocation, it displays bradycardia when the provocation grows severe. This cardiac ...rdation is likely to be part of a "freezing" reaction but, unlike opossums, the grouse ...ed to be very alert in its "frozen" state. Moreover when the provocation reached a ...ain magnitude (possibly when the bird got the impression of being detected) the brady-...dia vanished abruptly and tachycardia ensued. It is interesting that the domesticated ...se, which was familiar with man, did not exhibit such a reaction.

...ne can wonder what significance these cardiovascular manipulations might be of to ...bird. This question must, however await its solution since we at present are unable to ...ow the blood pressure and hence the true cardiovascular state of the bird, *i.e.* whether ...bradycardia is associated with a neurogenic vasoconstriction (as during a dive) or with ...bition of sympathetic vasoconstrictor fibre discharge (as in emotional fainting in man). ...fact that one of the hens maintained its bradycardia right up to the moment of take-off ...le one always switched to tachycardia prior to the flight nevertheless indicates that both ...y- and bradycardia are adequate preparations for quick flight.

...he shift from one cardiac state to the other can be compared to the very prompt shift ...autonomic cardiovascular pattern when a duck emerges from a dive: bradycardia and ...oconstriction shift within 1–3 s to intense tachycardia and vasodilatation.

...Inge Mohus and Mr Bård Holand, Department of Reguleringsteknikk, SINTEF Trondheim, contri-...ed significantly to the technical aspects of this study. We are indebted to colleagues in the field and in ...laboratory for valuable advice, particularly to Drs A. S. Blix and B. Folkow. The study was supported ...T Gelans Foundation and by a donation from Apotekernes Laboratorium.

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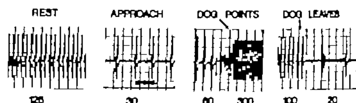


Fig. 1 A recording of the cardiac activity of a brooding, wild grouse hen (hen 1) during provocation by a bird d g. The numbers below the records give the heart rate in beats/min.

feathers (Bergerud *et al* 1963). The two hens (1 and) had 11 and 12 eggs respectively. We watched them daily until the eggs hatched This took 12 days for hen 1 and 8 days for hen 2.

An observation hut was raised about 70 m from each nest The hens could be closely followed in a telescope, a d the activities of other animals could be observed, since the landscape was open few scattered birches.

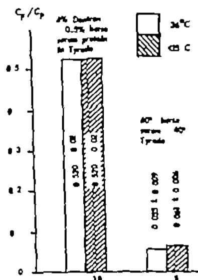
Two captive willow grouse hens were similarly equipped with transmitters. These birds were in captivity and were ccustomed to people. One of them lived with a cock and was brooding in a big door cage, the other st yed alone in her indoor cage.

The heart rate of the wild, brooding grouse hens was quite variable and sensitive to environmental disturbances When the hen was resting and undisturbed, her heart rate was between 120 and 140 beats/min. However when her mate uttered warning calls, when a redwing (*Turdus iliacus*) screamed upon leaving the nest or if a crow or raven passed the nest, her heart rate increased 20–30 % On other occasions we recorded a transient cardiac retardation of 20–30 %. This occurred when we coughed, yelled, broke off branches or a shot in the vicinity of the nest. In some cases the cardiac retardation was preceded by a short lasting cardiac acceleration.

The most dramatic cardiac response was recorded when we approached the brooding hen at a distance of 25 m or less. Thus, when a person, with or without dog, was seen by the hens they appeared to become very attentive as observed through the telescope. They were absolutely quiet (freeze ") barely blinking an eye. As the intruder approached her the cardiac activity invariably decreased and became irregular (Fig. 1) The degree of bradycardia increased with the intensity of the provocation the closer we came, the slower did the heart beat. At a distance of 2–4 m the heart rate was often down to 20–30 beats/min. On one occasion a heart rate of 45 beats/min, or 30 % of normal was maintained for 5 min of provocation.

When we stopped in front of a hen or the dog pointed at her she remained motionless. However the cardiac response was different for the two hens in this situation. Hen 1 immediately increased her heart rate 5- to 10-fold (Fig. 1) while the other maintained bradycardia unchanged. Once we moved away from hen 1 bradycardia resumed immediately and eased away as we walked off It is noteworthy that, in some cases, the heart rate changed by a factor of 10 from one beat to the next. Hen 2 maintained a low heart rate right until the moment she flushed. Both hens continued to breathe during the bradycardia, although we did not record the rate of breathing. These responses were tested repeatedly for both hens.

The captive birds never froze when we approached The single bird would become somewhat excited and her heart rate went up to about 200 beats/min. The brooding hen w



1. Illustrates how transcapillary passage of albumin is unaffected by even extensive reductions of cellular ... caused by tissue cooling. Thus, filtrate to perfusate concentration ratios of labelled albumin (C_F/C_P) are ... in "control" (36°C) and "test" (cooled below 15°C) ... of rats, as studied with two different perfusates. ... independent of tissue metabolism, C_F/C_P increases ... only however the perfusate contains dextran, as dealt ... in another study (Rippe and Folkow 1977). Since CF/Cp ... the horse serum perfusate is of the same order as in ... muscle in vivo (Appelgren, Jacobsson and Kjellmer ...), these results cannot be ascribed to any abnormal protein leakage.

... "test" preparation was kept well below 15°C, implying that its tissue metabolism was only a minor ... of that in the "control".

... steady state was reached in tissue temperatures the radioactive albumin was added to the common ... system and perfusion was continued for another 60 min. During this period venous pressures were ... to 11–12 mmHg inducing a modest and identical degree of filtration in both preparations since it ... mean capillary pressure about 8 mmHg (Rippe and Folkow 1977). Then the two hindquarter ... were washed free from tracer by perfusion for another 10 min at normal venous pressures ... the same perfusate but without tracer. Therefore, the only radioactive albumin now present in the preparations was that which had passed into the interstitial space. The perfusion was then interrupted, three pairs of corresponding muscle groups (gastrocnemius, quadriceps, tibialis anticus) were dissected and weighed, after which their radioactivity was measured in a well scintillation counter. Also the ... formation during the perfusion period could be estimated in both preparations by relating the wet weight of the dissected muscle groups to their dry weights, comparing these values with identical muscle ... taken from matched Wistar rats not used for experiments (*cf.* Rippe and Folkow 1977).

The essential results of this paired comparison of capillary protein transfer in skeletal muscle tissue of rats, at normal and markedly reduced tissue temperature and metabolism, are shown in Fig. 1. It is clear that the extent of radioactive albumin escape into the interstitial fluid space is essentially the same whether the hindquarter preparations had been throughout exposed to the normal tissue temperature of 36°C or had been kept cooled below 15°C. Neither did the two preparations differ concerning edema formation, though resistance to flow was higher in the cooled preparation in due proportion to the temperature-dependent increase of perfusate viscosity. Since this viscosity increase proportionally affects the resistance to flow in the pre- and post-capillary resistance sections, it implies a corresponding rise in mean capillary pressure during constant flow perfusions which, other things being equal, would increase the filtration rate. However, since also filtration across capillary walls largely obeys Poiseuille's law, it would be similarly influenced by the raised viscosity of the passing fluid. It may thus be expected that the filtration rate would remain largely ... in the two preparations and this was confirmed by the measured edema formation. With 4% Dextran with 0.5% horse serum protein, it amounted to 15.5 ± 1 ml/100 g · 60 min

Acta physiol. scand. 1977 100 258–260
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Is Capillary Micropinocytosis of any Significance for the Transcapillary Transfer of Plasma Proteins?

By

B Rippe, A Kashya and B. Folkow

It has been repeatedly suggested that micropinocytosis plays a decisive role in the tr of macromolecules across capillary walls. The evidence for this hypothesis is, however, indirect Thus physiological data (*e.g* Carter *et al* 1974 Renkin *et al* 1974) cannot ex that macromolecular transfer takes place by way of diffusion and/or filtration throug "large pores" (*cf* Landis and Pappenheimer 1963 Crone 1974). Ultramicroscopic pi (*e.g* Simionescu *et al* 1973 1975 Jennings and Florey 1967) no doubt illustrate t of macromolecular tracers within endothelial micropinocytotic vesicles, and their appearance in the interstitial fluid space. It should be stressed, however that such pi cannot alone be taken as proof of any directed *transendothelial* vesicle passage an opinions, also among experts on ultrastructure, seem to differ somewhat. The tracer ap ance in the interstitial fluid may for example, be the result of diffusion and/or fil through nearby pores which because they make out such a minor fraction of capillary are very difficult to visualize. In fact, the ultrastructural pictures might rather ill *e.g* an endothelial phagocytotic activity" of great importance in its own right but w relevance for active" *transcapillary* passage.

In recent studies on capillary permeability designed for concomitant measurem both filtration and diffusion events in various physiological states, the transcapillary t transfer process was analysed as well, using the artificially perfused rat hindquarters and Stage 1976 Rippe and Folkow 1977) This preparation mainly consisting of s muscle, also appeared suitable for exploring whether albumin transfer to any sig extent occurred by means of micropinocytosis. Particularly if implying a directed cellular passage, this process would call for some type of *active* cell mechanisms whic cerning rate and extent, must depend on the current level of cell metabolism.

An *in vitro* technique for paired constant flow perfusion of the isolated h ndquarters from two Wistar rats was used since it allows of precise control of circulatory event (*cf* Folkow *et al* 1 perfusates were used either 4 % Dextra in Tyrode solution with ddition of 0.5 % horse serum (10 paired expts.), or 60 % horse serum dil ted with Tyrode sol tion (5 paired expts.). To both pe (which were fully oxygenated) radioactive lbumin (2 10⁻⁸ g/l) was added f the course of the pe Perfusion pressure was measured in the can nlated ta l arteries, t il and paw being tied off su lymphatic drain ge, and both vascula beds were kept maximally dilated by addition of papavern

After 20 min of control perfusion t 36 C the temperat re of the perfusat t only one to the hind ("test") was reduced to below 5°C by precooli g device, wh le maintaining that to the other prep ("control") t 36 C. Thus, wh le tissue temperature of the control" remained normal through

On sabbathical leave from Inst Med. Dent Eng T kyo Med. & Dent. Univ J pan.

Acta physiol. scand. 1977 100, 261–263
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...sociation of Preovulatory Maturational Events in Rat Oocytes and Cumuli in the Presence of Dibutyryl Cyclic AMP

By

Torbjörn Hillensjö

...alian oocytes enter the first meiotic division during pre-natal life. Shortly thereafter ...ytes become arrested at the diplotene (dictyate) stage of the first meiotic division and ...main arrested until just prior to ovulation. The ovulatory LH-surge leads to resump-...' meiosis and the oocytes of preovulatory follicles undergo nuclear progression from ...e to metaphase II (oocyte maturation). Although it is generally agreed that LH is ...sible for the resumption of meiosis, the cellular mechanism for this hormonal effect ...s to be elucidated. The immature oocyte in the follicle is surrounded by specialized ...osa cells comprising the cumulus oophorus. Morphological (Zamboni 1970) as well ...hemical (Biggers *et al.* 1967 Gwatkin and Andersen 1976) evidences suggest a role ...cumulus cells in the regulation of both oocyte growth and maturation. Following the ...ory LH-surge the cumulus surrounding the maturing oocyte is radically changed into ...rsed structure of cells embedded in a mucopolysaccharide matrix (... Austin 1961). ...recent report isolated preovulatory follicles of PMSG-injected immature rats were ...d in an incubation system with chemically defined medium (Hillensjö *et al.* 1976). ...LH was added to the medium the follicle-enclosed oocyte resumed meiosis and the ...us was transformed into a dispersed structure which was sensitive to hyaluronidase. ...m of this study was to see whether dibutyryl cyclic AMP (dbcAMP) would mimic ...fects of LH on the follicle-enclosed oocyte itself and on the cumulus oophorus.

...ere Sprague-Dawley rats maintained under standardized environmental conditions (light 05^{00}–19^{00} h) ...jected ... in the morning of day 28 ...ith 8 IU PMSG (Gestyl ... Organon). This treatment leads to ...on in the early morning of day 31 ...ith ... physiological number of ova (Hillensjö *et al.* 1974, ...al 1976). In this study the rats were killed by cervical dislocation in the forenoon of day 30 and ...latory ovarian follicles isolated by micro dissection. Ten–twelve follicles from each animal ... were ...ted in ... chemically defined medium (Hillensjö *et al.* 1976) either plain or containing the test sub-...r Ovine LH (NIH-LH-S18 10 µg/ml), dbcAMP (Sigma, 1.0 mM) or theophylline (Sigma, 5.0 mM) ...cated below. After incubation for 6 or 10 h the oocytes ...ith their cumuli were recovered from the ...s for Nomarski interference contrast microscopy. The oocytes were classified as: immature (con-...: the germinal vesicle ...ith its nucleolus), oocytes with germinal vesicle breakdown (GVB) and oocytes ...rst polar body (PB). The cumuli ...ere arbitrarily classified as: compact cumuli with smooth outline ...act"), compact cumuli ...ith irregular outline ("rough") and dispersed cumuli in ... viscous matrix ...rsed"). Furthermore the lysing effect of testicular hyaluronidase (Leo Ltd, 15 000 IU/ml) was ...d as described in detail before (Hillensjö *et al.* 1976). The cumuli ...ere then classified as non-sensitive ...rtially sensitive ( ), or fully sensitive (+) to hyaluronidase.

at 36 C and to 14 4 ± 0 6 ml/100 g × 60 min at below 15°C. With the diluted horse se edema formation was 32.4 ml and 28 8 ml, respectively colloid osmotic pressure bei considerably lower than for the Dextran perfusate.

However the rate and extent of such components of micropinocytosis that are dep on cellular metabolism must have been markedly depressed in the cooled hindquarter ration Since nevertheless the albumin transfer across the capillary walls was found essentially the same in the "test" and "control" preparations in all the fifteen paired ments, it appears as if micropinocytosis in the capillary endothelial cells, importan may well be for other processes, is *not* responsible for the normal passage of plasma p from blood to the interstitial fluid passage.

An alternative would be that both the creation of pinocytotic vesicles and transendo passage by such means are entirely *passive* physical processes. Even though such an al tive cannot perhaps be entirely ruled out, it seems highly unlikely for several reas would, for example, be difficult to explain why particularly histamine and bradyki greatly increase plasma protein transfer in case this transfer were based on entirely micropinocytotic processes.

Against such a background it seems reasonable to conclude from the present finding the transcapillary passage of plasma macromolecules is essentially based on diffusi filtration either through intercellular "large pores or/and through "pores est across the endothelial cells by random confluences of micropinocytotic vesicles, as il illustrated in electron microscopic pictures by Simionescu, Simionescu and Palade (1

This paper was supported by the Swedish Medical Research Council (Project No B 77-14x-00 and grant from the Faculty of Medicine, University of Göteborg. Thanks are due to Mrs Gertrud K for skilful and devoted technical assistance.

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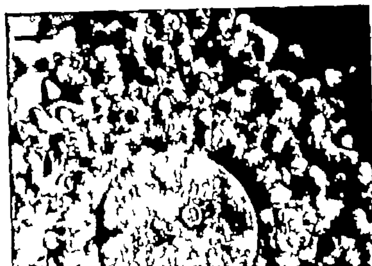
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...otomicrograph of ... cumulus-enclosed oocyte recovered from ... follicle that had been incubated ...n the presence of LH and theophylline. Note the presence of the germinal vesicle containing ...t nucleolus and the cellular dispersion of the cumulus. Scale 20 μm.

...e thanks Professor Kurt Ahrén and Docent Lars Hamberger for valuable criticism and Mrs ...Thelander for expert technical assistance. LH S18 was kindly supplied by the NIH, USA. The ...s supported by grants from the Ford Foundation (760-0087), the Medical Faculty University ...org and Handlanden Hjalmar Svenssons Forskningsfond.

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TABLE I Effect of LH dbcAMP and theophylline on follicle-enclosed oocytes and cumuli

Incubation time	Medium	Oocytes		Cumuli		
		% GVB	% PB	structure	lysis	
6 h	Control	6	0	compact	0	[illegible]
	LH	98	0	rough-dispersed	+	[illegible]
	dbcAMP	19	0	rough	0 +	[illegible]
	dbcAMP + LH	13	0	rough-dispersed	+	[illegible]
	theophylline	0	0	compact	0	[illegible]
	theophylline + LH	0	0	rough-dispersed	0 +	[illegible]
10 h	Control	16	0	compact	0	[illegible]
	LH	100	60	dispersed	+ +	[illegible]
	dbcAMP	37	0	dispersed	+ ++	[illegible]
	dbcAMP + LH	41	0	dispersed	+ +	[illegible]
	theophylline	0	0	compact-rough	0 +	[illegible]
	theophylline + LH	0	0	dispersed	+ +	[illegible]

Preovulatory follicles were extirpated in the morning of day 30 from immature rats pre-treated with The follicles were incubated for 6 or 10 h either in plai med um or in medium containing dbcAMP o theophylline 5 mM with or without LH 10 μg/ml. After incubation the oocytes were morpho examined and the percentage with GVB or PB of the total number (n) calculated. The structure cumulus and the lysing effect of hyaluronidase is given [(0) non-sensitive, (+) partially sensitive (fully sensitive.]

The results are summarized in Table I In confirmation with an earlier report (Hillensjö 1976) LH induced oocyte maturation and cumulus transformation, the effects being pronounced after 10 h of incubation In the presence of dbcAMP a small proportion 37%) of the oocytes showed GVB but no PB were seen. The nucleotide mimicked the of LH on the cumuli which were transformed into dispersed, hyaluronidase-sensitive tures. In the combined presence of LH and dbcAMP oocyte maturation was largely prev whereas the cumulus transformation occurred unimpaired. It was also found that theo line completely blocked the meiosis-inducing action of LH without impairing the hor effect on cumulus transformation (Table I Fig. 1)

Comments. Several actions of LH in the periovulatory follicle are believed to be me by cAMP *e g* on steroidogenesis (Marsh 1976), luteinization (Kolena and Channing and ovulation (Rondell 1974) This study indicates that also the gonadotrophic eff the differentiation of the preovulatory cumulus is mediated by cAMP The role of in oocyte maturation is less clear Tsafriri and co-workers (1972) found that the inject dbcAMP into the antrum of cultured preovulatory rat follicles resulted in oocyte matu On the other hand it was shown that dbcAMP reversibly inhibits the spontaneous tion of cultured mouse oocytes (Cho *et al.* 1974) Further work is needed to establish w cAMP is indeed directly involved in oocyte maturation The fact that the maturational in the oocyte and the cumulus are dissociable suggests that the two celltypes of this co although functioning as a unit, are regulated through different biochemical adjust in their periovulatory differentiation

t secretion of ³H NA evoked by identical stimulation of the nerves of guinea-pig eferens initially seemed to follow a closely similar course (Fig. 1 A), tending to level s the stimulus strength rose to 50 V (current between electrodes, 69 mA). However ther increase in the strength of the applied shocks caused the secretion of H-NA to gain, more steeply. No well-defined 'supramaximal' level of stimulation was reached n the range tested. In view of the recent evidence that vas deferens (at least in the rat) be supplied by two sets of adrenergic fibres, with different pharmacological properties can and McGrath 1976), one possible interpretation of these results is that the initial n ³H-NA secretion was due to selective activation of low-threshold fibres, while the d rise would be due to progressively increasing recruitment of a different set of fibres. e of these would in that case have to have a very high threshold of excitation, since they not appear to be activated even by the strongest shocks used (120 V on the Grass stimu- 165 mA between the electrodes).

o further analyze the excitability of the adrenergic nerves of the two preparations, they stimulated with shocks of a constant high strength (120 V) but of varying duration (from to 10 ms). Again the profile describing the ³H-NA secretion from the human vasomotor es (Fig. 1 B) suggested that these fibres, with respect to excitability represented a single ogeneous population 'supramaximality' seemed to be approached as the duration of ks went beyond 0.3 ms. The curve describing the rise in H NA secretion from vas rens, with increasing duration of stimulus shocks, was strikingly different (Fig. 1 B). tw straight lines obtained in the semilog plot of ³H-NA secretion against duration of ks suggested that the current of excitation had evoked two saturable processes, one ment below 1 ms and one at durations from 1 to 10 ms. This might be regarded as itional support for the existence of two different sets of adrenergic nerve fibres in vas rens (Duncan and Mc Grath 1976), one with low and one with very high treshold for trical excitation.

n alternative interpretation is that the initial rise in H NA secretion, with increasing ngth and/or duration of stimulus shocks, reflected increasingly complete recruitment ll the nerve fibres of the preparation, and that the second rise in ³H NA secretion was result of a direct facilitating effect of the exogenous current on the secretory mechanisms. ce the ³H NA secretion evoked by field stimulation, even at 120 V and 10 ms, was almost mpletely abolished with tetrodotoxin (0.1 μg/ml), in both preparations, the direct depolariz effect of the current of stimulation on the varicosities was clearly not sufficient to initiate retion. But this does not exclude the possibility that it could facilitate secretion in varicosi- already invaded by propagated impulses generated by an action of the current of stimula n on preterminal parts of the axons. This interpretation of the present data is strongly pported by the finding of a common V_{max} level for the secretion of ³H NA in guinea- vas deferens, on kinetic analysis of its calcium dependence when evoked by field stimula n at either 60 V or 120 V (Stjärne, to be published).

In conclusion. Judging excitability on measurement of the secretion of H-NA evoked field stimulation, the 'long' adrenergic vasomotor fibres of human blood vessels appear represent a single, homogeneous population. This is less clearly the case for the 'short' renergic nerv fibres of guinea-pig as deferens. The dual secretory response in these

Acta physiol. scand. 1977 100. 264-266
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Differences in Secretory Excitability between Short and Long Adrenergic Neurons: Comparison of ³H Noradrenaline Secretion Evoked by Field Stimulation of Guinea Pig Vas Deferens and Human Blood Vessels

By

LENNART STJÄRNE

On comparing the secretion of noradrenaline (NA) evoked by electrical field stimulation of the short adrenergic nerves of guinea-pig isolated vas deferens (Sjöstrand 1962) with that evoked by similar stimulation of the long adrenergic nerves of human isolated arteries and blood vessels, using identical experimental conditions, it was found that the two types of nerve differ markedly in secretory excitability While it was possible to adjust the strength and/or duration of stimulus shocks to a level causing a well-defined maximum in ³H-NA output from the human vasomotor nerves no such supramaximal level could be reached in the nerves of guinea pig vas deferens

The methods used for setting up superfused field stimulated strips of human arteries and veins, or of guinea-pig isolated vas deferens, have been described in detail elsewhere (Stjärne and Brundin 1976 Stjärne 1977) The tissue stores of NA were labelled by preincubation with 10 μCi/ml (about 400 ng) of ³H-(−)-NA (New England Nuclear Corp). The superfusion medium (Tyrode solution aerated with 6.5% CO_2 in O_2, temperature was 37°C and flow rate 2.5 ml/min) contained 0 6 μM desipramine and 10 μM normetanephrine to prevent tissue binding of NA released from the neural stores. The strips were stimulated electrically via two platinum ring electrodes, one at the bottom and one at the top of the preparation (distance between electrodes 40 mm), using a Grass S44 stimulator Pulses were biphasic· the voltage on the stimulator was varied from 10 to 120 V and the resulting voltage and current between stimulation electrodes were displayed on a Tektronix Type 502A oscilloscope Duration of pulses was varied between 0.01 and 10 ms. The secretion of NA evoked by nerve stimulation was monitored by measuring the evoked fractional rise in efflux of ³H NA (rise in efflux of ³H divided by total ³H in the tissue at the time of stimulation, *cf* Stjärne and Brundin 1976)

On stimulation with trains of 300 shocks at 10 Hz (duration 2 ms) the secretion of ³H-NA from the human vasomotor nerves rose with the current between the stimulation electrodes (Fig. 1 A) 'Supramaximality' seemed to be reached at 165 mA (120 V on the Grass stimulator) Judging from the shape of the curve it seems likely that this level represented the threshold for generation of propagated impulses in the least excitable fibres of the preparation.

retion of ³H-NA evoked by identical stimulation of the nerves of guinea-pig ns initially seemed to follow a closely similar course (Fig. 1 A), tending to level stimulus strength rose to 50 V (current between electrodes, 69 mA). However, increase in the strength of the applied shocks caused the secretion of ³H-NA to , more steeply. No well-defined 'supramaximal' level of stimulation was reached range tested. In view of the recent evidence that vas deferens (at least in the rat) upplied by two sets of adrenergic fibres, with different pharmacological properties and McGrath 1976), one possible interpretation of these results is that the initial I-NA secretion was due to selective activation of low-threshold fibres, while the e would be due to progressively increasing recruitment of a different set of fibres. these would in that case have to have a very high threshold of excitation, since they ppear to be activated even by the strongest shocks used (120 V on the Grass stimu- 3 mA between the electrodes).

ther analyze the excitability of the adrenergic nerves of the two preparations, they ulated with shocks of constant high strength (120 V) but of varying duration (from 0 ms). Again the profile describing the H NA secretion from the human vasomotor Fig. 1 B) suggested that these fibres, with respect to excitability represented a single neous population. 'supramaximality' seemed to be approached as the duration of ent beyond 0.3 ms. The curve describing the rise in ³H NA secretion from vas , ith increasing duration of stimulus shocks, was strikingly different (Fig. 1 B). straight lines obtained in the semilog plot of H NA secretion against duration of uggested that the current of excitation had evoked two saturable processes, one t below 1 ms and one at durations from 1 to 10 ms. This might be regarded as al support for the existence of two different sets of adrenergic nerve fibres in vas (Duncan and he Grath 1976), one with low and one with very high treshold for l excitation.

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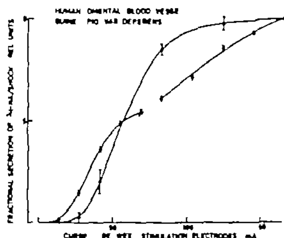


Fig. 1 A

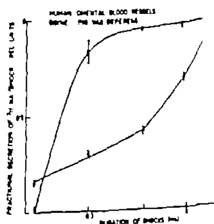


Fig. 1 B.

Fig. 1 A. H NA secretion as a function of the intensity of stimulus current Field stimulation 1 m ... shocks at 10 Hz. The values normalized with respect to the maximum values obtained, in each prepn (at 165 mA) Data from human omental blood vessels Means and range (n = ?), from ... deferens. Means ± S.E. (n = 4–5 on each point).

Fig. 1 B H NA secretion as a function of the duration of shocks. Field stimulation at 165 mA, ... shocks at 10 Hz. The values normalized with respect to the maximum values obtained, in each prepn. Note that while for the blood vessel preparations the ordinates of Fig. 1 A and B are almost ... (1 0 in Fig. 1 A corresponding t 0 97 i Fig 1 B), they differ considerably for vas deferens (1 0 in Fig ... corresponding to 0.60 in Fig 1 B). Data from human blood vessels Means and range (n ...), from ... deferens Means ± S.E. (n = 4–5 on each point).

fibres may indicate that the exogenous current of the field stimulation in addition to ... ing propagated nerve impulses also facilitated secretion by an action on varicosities ... invaded by nerve impulses This may be the reason why it was not possible, with the electr... and stimulation parameters used, to reach a well-defined maximal secretory response ... responding to 'supramaximality of stimulation, in the vas deferens preparation.

This work was supported by grants from the Swedish Medical Research Council (project B77-04... 03C), Magnus Bergvalls Stiftelse and Karolinska Institutets Fonder I thank Mrs Eivor Stjärne and I ... Eriksso for excellent technical assistance.

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Acta physiol. scand. 1977. 100. 267–269

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...staglandin E_2 and α or β-Adrenoceptor Mediated Interferences ... ^{3}H-Noradrenaline Secretion from Human Vasomotor Nerves: ...Comparison between Effects on Omental Arteries and Veins

By

LENNART STJÄRNE and JAN BRUNDIN

...ier ...ork with human vasomotor nerves (Stjärne and Gripe 1973) it has been shown ...e inhibition of ^{3}H-NA secretion by exogenous NA, and the enhancement caused by the ...noceptor antagonist phentolamine, occur even after blocking formation of prosta...n E_2 (PGE_2) with 5,8,11,14-eicosatetraynoic acid (ETA). This shows that at least part of ...adrenoceptor mediated control of NA secretion is independent of (not mediated by) ...enous PGE_2. In the same paper it was also shown that the inhibitory effect of exo...s PGE_2 on ^{3}H-NA secretion persists in the presence of phentolamine, indicating that ...nous PGE_2 acts on a target in the nerves different from that of NA (Stjärne 1973). ...as also been found that the enhancement of the secretion of ^{3}H NA caused by the ...ent isoprenaline (Stjärne 1975, Stjärne and Brundin 1975) occurs even after blocking ... formation with ETA. This indicates that β-receptor mediated stimulation of the ...ory mechanism was not, or was not entirely due to interference with the local forma... of PGE_2 (Stjärne and Brundin 1976 a).

...e present paper reports results from continued analysis of possible interaction between ...fferent mechanisms controlling ^{3}H NA secretion from the vasomotor nerves of human ...tal arteries and veins, which were recently found to differ with respect to innervation ...e smooth muscle. The nerves of the arteries were restricted to a layer between the ...a adventitia and tunica media, but those of the veins penetrated into the smooth ...le layer of the tunica media (Thureson-Klein *et al.* 1976). In view of this it was of ...est to compare the mechanisms of control of ^{3}H NA secretion in the adrenergic nerves ...e omental arteries with those in the nerves of the corresponding veins.

...e experiments were carried out using 11 preparations of omental blood vessels (4 ...ries and 7 veins), of a length of about 4 cm and an external diameter of 1–2 mm, obtained ...biopsy specimens during abdominal surgery under general anesthesia, performed on 6 ...ale patients 30–47 years old (1 Caesarian section and 5 cases of laparotomy for various ...r reasons). The methodology of superfusion, nerve stimulation and determination of ...NA secretion per shock was identical to that described by Stjärne and Brundin (1976 a).

...reduce the scatter of data the fractional secretion of ^{3}H NA (Fig. 1) is presented in ...the units the values for ^{3}H-NA secretion per shock in the individual experiment were ...malized with respect to the average of the values obtained during the 2 stimulation ...iods following infusion of ETA in that same experiment (this average was given the value

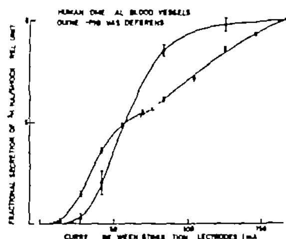


Fig. 1 A

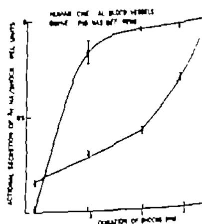


Fig. 1 B.

Fig. 1A H NA secretion as a function of the intensity of stimulus current. Field stimulation ... shocks at 10 Hz. The values normalized with respect to the maximum values obtained, in each prep... (t 165 mA). Data from human omental blood vessels Means and range (n = 2) from ... deferens. Means ± S.E. (n = 4–5 on each point).

Fig. 1B H NA secretion as a function of the duration of shocks. Field stimulation at 165 mA, ... shocks at 10 Hz. The values normalized with respect to the maximum alues obtained, in each prepa... Note that while for the blood vessel preparations the ordinates of Fig 1A and B are almost iden... (1 0 in Fig. 1A corresponding to 0.97 in Fig. 1B), they differ considerably for vas deferens (1 0 in Fig... corresponding to 0.60 in Fig. 1B). Dat from human blood vessels Means and range (n 2), ... deferens Means ± S.E. (n = 4–5 on each point).

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ion appeared to increase secretion in the veins more than in the arteries (by an average and 61%, respectively). The difference was not statistically significant, however 1), possibly due to the limited number of observations (5 veins, 2 arteries).

tendency towards differences in the responses of the nerves of arteries and veins was en on infusion of PGE_2 (Fig. 1). The dose-dependent depression of ^3H-NA secretion so practically the same in the presence of isoprenaline and after ETA as before the on of these drugs.

results indicate that there may exist certain differences in the effects of drugs on the ion of NA from the adrenergic nerves of human omental arteries and veins (secondary tomically based differences in accessibility?). The combined effect of isoprenaline + was about the same as that previously observed when these agents were added in verse order (Stjärne and Brundin 1976 a). The results therefore indicate that the facili- effect of β_2-adrenoceptor stimulation may be normally restricted by formation of enous PGE_2. The level reached with this dose of isoprenaline after ETA may thus ent a 'ceiling' to the β_2-adrenoceptor-mediated facilitation of the secretory process ne and Brundin, to be published).

e results also show that the inhibitory effect of exogenous PGE on ^3H NA secretion ted with equal efficiency in the presence as in the absence of β_2-receptor stimulation of treatment with an inhibitor of PGE synthesis. This suggests that isoprenaline and did not compete for the same site in the mechanisms controlling ^3H NA secretion. enhancing effect of phentolamine on the secretion, even in the presence of isoprenaline after ETA indicates that the α-adrenoceptor mediated inhibitory effect of endogenous (which has earlier been shown not to be mediated by endogenous PGE_2, Stjärne and e 1973) is not, or not to any important extent, directed towards the same target in the tory machinery as that of the β_2-adrenoceptor mediated facilitation.

rk was supported by grants (to L. S.) from the Swedish Medical Research Council (project B77 03027-08C), from Magnus Bergvalls Stiftelse and from Karolinska Institutets Fonder. We thank Eiver Stjärne and Mrs. Ingamarie Eriksson for excellent technical assistance.

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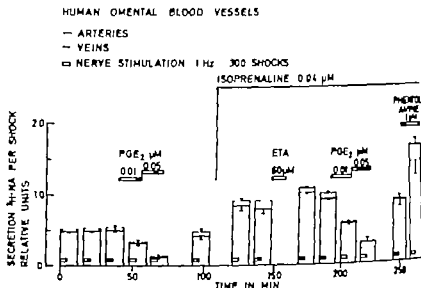


Fig. 1 Fractional secretion of ³H NA in response to nerve stimulation. Effect of drugs. Mean ± (n = 4 7). For details see the Text

of unity) During the initial 3 control stimulations with trains of 300 shocks at 1 Hz evoked secretion of H NA per shock remained constant (Fig. 1), at an average value of $(2.41 \pm 0.10) \times 10^{-4}$ (n = 29) for arteries and $(2.85 \pm 0.14) \times 10^{-4}$ (n = 33) of the tissue content of ³H-NA The slightly higher value in the veins ($p < 0.05$) could be due to differences in diffusion conditions (*cf* above about differences in neuromuscular relationship in arteries and veins, Thuresson-Klein *et al.* 1976), and therefore degree of tissue binding of H NA secreted from the nerves. But on the other hand μM desipramine and 10 μM normetanephrine added to the medium should tissue binding. Thus the higher evoked rise in efflux of H NA in veins may reflect differences in fractional secretion of NA from vasomotor nerves in arteries and veins.

The effects of drugs tended to support the general impression of certain differences in 'reactivity' of the mechanisms for H NA secretion in the nerves of arteries and veins. Maximal stimulation (Stjärne and Brundin 1975) of the β adrenoceptors of the preparation (Stjärne and Brundin 1976 b) with 0.04 μM isoprenaline enhanced the secretion of ³H to the same extent in arteries and veins, by 89.5 and 90% respectively The enhanced was better maintained in the arteries while it tended to drop slightly in the veins (by average of 11.6% in the second period during isoprenaline, $p > 0.05$) Partly because of subsequent infusion of the irreversible blocker of PGE₂ synthesis ETA appeared to cause much more marked further rise in H NA secretion in the veins. The overall rise in NA secretion by the combined effect of β_2-receptor stimulation and blocking of formation endogenous PGE (the level reached immediately after ETA compared with that before isoprenaline) was 166 ± 28.7% in the veins and 110.0 ± 7.5% in the arteries (p As after the beginning of isoprenaline infusion the enhanced level of secretion was well maintained in the second period after ETA in the veins than in the arteries (Fig Addition of 1 μM phentolamine to remove α-adrenoceptor-mediated restriction of ³H

For this comparison data from 6 additional patients were included.

on appeared to increase secretion in the veins more than in the arteries (by an average and 61 %, respectively). The difference was not statistically significant, however 1), possibly due to the limited number of observations (5 veins, 2 arteries).

tendency towards differences in the responses of the nerves of arteries and veins was on infusion of PGE (Fig. 1). The dose-dependent depression of ³H NA secretion ho practically the same in the presence of isoprenaline and after ETA as before the on of these drugs.

results indicate that there may exist certain differences in the effects of drugs on the ion of NA from the adrenergic nerves of human omental arteries and veins (secondary atomically based differences in accessibility?). The combined effect of isoprenaline + was about the same as that previously observed when these agents were added in verse order (Stjärne and Brundin 1976 a). The results therefore indicate that the facili- g effect of β_1-adrenoceptor stimulation may be normally restricted by formation of genous PGE_2. The level reached with this dose of isoprenaline after ETA may thus ment a ceiling to the β_1-adrenoceptor-mediated facilitation of the secretory process rne and Brundin, to be published).

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Acta physiol scand. 1977 100 270-272
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Structural "Resetting" of the Renal Vascular Bed in Spontaneously Hypertensive Rats (SHR)

By

B Folkow G Göthberg S Lundin and S E. Ricksten

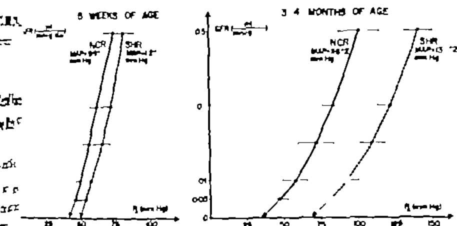
Studies in both man and rats (*cf* Folkow *et al* 1973 1974) show that a rapid struc adaptation occurs early in hypertension which, besides the left heart and systemic arte affects the precapillary resistance vessels in a hemodynamically most important way a wall (mainly media) thickening takes place which tends to encroach upon the inner ra thus raising the w/r_i ratio The rapidity generalization and extent of this precapillary "st tural autoregulation" to average changes in pressure load makes it crucial both for initiation and maintenance of a chronic high-pressure state, whichever the initiating fac are The same principle of hypertrophic adaptation in the left heart and large arteries l to a functionally important displacement of the Starling curve towards the right (W bäck, Isaksson and Noresson 1975) and to baroreceptor resetting (*e.g* Jones 1977).

The question arises how this principle of structural adaptation, *per se* a normal b response to average changes in load, affects the renal vascular bed. As amply illustrat Guyton *et al* (1974) the kidney particularly its vascular bed, must participate in the ge resetting of systemic resistance if persistent hypertension should ensue, whichever the m anisms of initiation and maintenance. An earlier study on adult SHR, in which neuro monal pressor influences appear to constitute a major trigger" mechanism, revealed the renal resistance vessels display the same characteristic hyperreactivity as in other circ suggesting a considerable media thickening (Folkow *et al* 1971) However at maxi dilatation and artificial perfusion the renal resistance to flow appeared to be lower in S than in normotensive controls (NCR)

Concerning renal function and re-setting the ratio of the pre and postglomerular re ances is, however far more relevant than total renal resistance, because the higher this r the lower the glomerular filtration Therefore if a structurally based increase of this r occurs a most efficient resetting of the renal "longterm barostat" would ensue and al the same principles that reset systemic resistance in general, the "short term barostats" the left heart. This problem was recently approached with a "stopflow technique" wh ureter pressure was raised until urine formation barely ceased in perfused, maximally v dilated SHR and NCR kidneys (Göthberg *et al* 1976) The results lent strong suppor the view that the pre to postglomerular resistance ratio is, indeed, considerably increase established SHR hypertension The problem is here approached from another angle.

Methods

Paired experiments were performed on two groups of SHR and ge-matched NCR. In the first (9 p SHR was in the "borderline hypertensive age of 5 weeks, where wah pressures (M



The relationship between arterial perfusion pressure (P_A) and glomerular filtration rate (GFR) in g dry weight) in the maximally vasodilated, artificially perfused kidneys from young (left part) and adult (right part) SHR and NCR. — Had the impact of "passive autoregulation" been corrected for, this would have made both the SHR and NCR curves much steeper but without appreciable changes of their interrelationships.

112±2 and 95±2 mmHg, respectively. In the second group (n = 11 pairs), with SHR in the early established phase of hypertension (3–4 months) MAP were 139±2 and 116±2 mmHg, respectively (for some reason MAP in NCR was here some 10 mm higher than usual).

The isolated vascular beds of one SHR and one NCR kidney were perfused in parallel via the aorta with oxygenated Tyrode solution containing 2 % Macrodex. The perfusate also contained nitroprusside sodium to ensure maximal renal vasodilatation and Cr-EDTA, 2 mM, for estimation of glomerular filtration rate (GFR) from the spectrophotometrically determined concentrations in perfusate and urine.

Perfusate flow to the two kidneys could by a pump be set at any desired level and arterial inflow pressure was continuously measured, the outflow leaving through the widely opened renal veins. Besides P_A and flow, urine formation in each kidney was continuously measured by a sensitive weighing device. The preparation was kept at room temperature to minimize tubular reabsorption, which generally was only 30–40 per cent and equal in both. — Perfusion was started at such low P_A levels that no glomerular filtration occurred. By stepwise P_A increases it was then noted how urine formation was related to P_A in NCR and SHR. In this way a curve, relating P_A to GFR, could be constructed for each kidney.

The relationship between P_A and flow reflected total renal resistance but was, with this perfusate and tubular reabsorption, considerably affected by "passive" autoregulation (due to tissue pressure increases, mainly caused by glomerular filtration and the consequent tubular distension), which masks the true vascular resistance. To reveal the latter in NCR and SHR, the opposite kidneys were perfused with kerosene, which does not pass across capillary walls, hence largely eliminating the pressure-flow curve distortion caused by "passive" autoregulation (Waugh 1958).

Results with Comments

The relationships between P_A and GFR in the maximally vasodilated NCR and SHR kidneys are shown in Fig. 1. It is clear (left part) that in the young, "borderline hypertensive" SHR the glomerular filtration curve just tends to be displaced to the right of the NCR curve, the difference being statistically not significant. However in established SHR hypertension the SHR glomerular filtration curve is displaced 30–40 mmHg to the right of the NCR one (right part). — The largely parallel displacement of the glomerular filtration curve in SHR strongly suggest that at these ages NCR and SHR hardly differ concerning glomerular filtration capacity. Had this been the case the two curves should differ also in steepness.

With maximally dilated vessels and the same perfusate these results can only be exp... as reflecting a gradual development in SHR of a structurally based increase of the pre... postglomerular resistance ratio This is established in close parallel to the similarly p... changes in *e.g* hindquarter precapillary resistance and in left ventricular weight, each reflecting secondary tissue adjustments in SHR to neurohormonal pressor influences ... are particularly prominent in early life Thus, concerning rate and extent of "struc... autoregulation" the renal preglomerular vessels correspond to the systemic precap... resistance vessels, just as they are each other s counterparts in functional autoregula...

Per unit kidney weight total renal vascular resistance, as deduced from the ... pressure-flow curves, was 15–20 per cent higher in adult SHR than in NCR. For the ... Tyrode perfusate the curves were, however reversed thanks to a far more pronounced ... autoregulation in NCR, which *per se* indicates an increased pre-/postglomerular resi... ratio in SHR, here serving to delimit the increases in capillary pressure, and hence in ... pressure during P_A rises. However even in adult SHR NCR the difference in "true ... resistance to flow was so modest that the increased preglomerular resistance in SHR ... well be associated with a lower postglomerular resistance than in NCR.

Clearly the proposed gradual alteration in the pre to postglomerular resistance ... SHR in response to pressure load implies an efficient structurally based re-setting ... of the renal "long term barostat function in nature closely related to the secondary ... tural resetting of the short term barostats of the left heart and of the precapillary re... ance vessels in general (Folkow *et al* 1973 1974) Thanks to the preglomerular w/r ... and consequent vascular hyperreactivity in SHR the renal vascular smooth muscles ... adjust blood flow and glomerular filtration even better than in NCR, at least before ... vascular degeneration interferes

Supported by the Swedish Medical Research Council (14X-00016) and by a grant from the Fac... Medicine, U iversity of Goteborg. Thanks are due to Mrs Ulla Axelsson and Mrs Birgitta Karlss... sk lful technical assistance.

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Acta physiol. scand. 1977 100 273–281

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Cerebral Blood Flow and Oxygen Consumption in Rat, Measured with Microspheres or Xenon

By

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Received 3 September 1976

Abstract

…DE A., S. M. DE LA MONTE and J. J. CARONNA. *Cerebral blood flow and oxygen consumption in rat measured with microspheres or xenon.* Acta physiol. scand. 1977 100 273–281

…erebral blood flow and, in some rats, the cerebral rate of oxygen consumption were measured in … groups of male rats. Fractionation of radioisotope-labeled microspheres was used to measure …al cerebral blood flow in four parts of the rat brain. The arterial and cerebral venous concentrations …dioactive xenon during desaturation were used to measure the blood flow and oxygen consumption …ates when venous blood was collected from the superior sagittal sinus, or of whole brain when the …verse sinus was sampled. The regional cerebral blood flow measured with microspheres had a large …dard error reflecting the technical difficulty of this method. The cerebral blood flow measured with … was higher when venous blood was sampled from the superior sagittal sinus than when sampled … the transverse sinus, but cerebral oxygen consumption rates were smaller. The difference reflects the …er trauma involved in the superior sagittal approach and possible extracerebral contamination present … the transverse sinus approach.

…the course of studies on cerebral metabolism in metabolic encephalopathy we evaluated …methods that determine cerebral blood flow and metabolism in the rat. Two methods were …posal. (1) The indicator fractionation of cardiac output with labeled plastic spheres …(Bendell and Hollenberg 1971, Sasaki and Wagner 1971), and (2) the Kety-Schmidt inert …dicator exchange method applied to the superior sagittal sinus of the rat brain (Eklof … *et al.* 1973, Norberg and Siesjo 1974 a, 1974 b) for the measurement of predominantly …rtical blood flow. A third method was developed for the measurement of whole-brain …ood flow and oxygen consumption with the Kety-Schmidt inert indicator exchange …ethod, modified by Scheinberg and Stead (1949) and applied to the transverse sinus of the …t brain (Gjedde *et al.* 1975).

The evaluation was designed to disclose whether cerebral blood flow and oxygen consumption rates determined by different methods agree when the determinations are performed under similar circumstances.

With maximally dilated vessels and the same perfusate, these results can only be ry[illegible] as reflecting a gradual development in SHR of a structurally based increase of the postglomerular resistance ratio This is established in close parallel to the similarly p[illegible] changes in e.g hindquarter precapillary resistance and in left ventricular weight, [illegible] reflecting secondary tissue adjustments in SHR to neurohormonal pressor influences are particularly prominent in early life. Thus, concerning rate and extent of "tru[illegible] autoregulation" the renal preglomerular vessels correspond to the systemic precap resistance vessels just as they are each other s counterparts in functional autoregulat[illegible]

Per unit kidney weight total renal vascular resistance, as deduced from the pressure-flow curves, was 15–20 per cent higher in adult SHR than in NCR. For the d[illegible] Tyrode perfusate the curves were however reversed thanks to a far more pronounced [illegible] autoregulation in NCR, which *per se* indicates an increased pre /postglomerular res[illegible] ratio in SHR here serving to delimit the increases in capillary pressure, and hence [illegible] pressure during P_A rises. However even in adult SHR NCR the difference in "true resistance to flow was so modest that the increased preglomerular resistance in SH[illegible] well be associated with a lower postglomerular resistance than in NCR.

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WAUGH, W H Flow a function of arterial pressure in the oil perfused kidney *Circulat Res.* 107 115

ch Q = tissue concentration of indicator, c_a = arterial concentration of indicator at time t, and cerebral venous concentration of indicator at time t.

... tissue concentration of microsphere-bound radioactivity was determined by gamma-counting. ... tissue concentration of xenon was calculated from the partition of xenon between cerebral venous ... brain tissue at equilibrium just prior to decapitation.

... gration of the radionuclide concentration difference was performed from concentrations measured in arterial and venous blood sampled intermittently from the appropriate catheters or mechanically ... difference between the mean concentrations of arterial and venous blood sampled at constant rate ... the catheters.

... Suspensions of microspheres in saline were obtained from the 3M Company. Diameters were ... by the supplier. However, previous studies have noted large variations in individual sphere ... (Prosser 1972). The microspheres were completely trapped in cerebral vessels, thus requiring ... only of arterial blood.

... Ce microspheres with a specific activity of 12.28 mCi g^{-1} and specified diameters of 15±5 μm ... diluted 1:10 with 4% w v^{-1} dextran, as were [^{85}Sr] microspheres with specific activity of 13.63 g^{-1} and specified diameters of 50±10 μm. Approximately 100 000 spheres in 100 μl were injected at cerebral blood flow determination.

... test tube filled with Extra Heavy Mineral Oil (Squibb) was connected to the tail artery catheter and Harvard withdrawal pump, calibrated to collect 256 μl min^{-1} of blood under the mineral oil. ... thus ... it was possible mechanically to integrate the radionuclide concentration curve of microspheres in ... blood, and CBF (ml 100 g^{-1} min^{-1}) was calculated from the following equation:

$$\mathrm{CBF} = \frac{(Q_r)/w}{T_{a_{sm}}/0.256} \cdot 100$$

... which Q_r and w = total radioactivity and weight (g), respectively, of brain tissue sample, and $T_{a_{sm}}$ = total ... activity of arterial sample.

... Cardiac output was similarly determined in one rat by using the total radioactivity of the injected ... microspheres instead of Q_r/w (cf. Passmore and Lasseck (1973)).

... Mechanical withdrawal of arterial blood began immediately prior to injection of the microspheres into ... cardiac catheter. The microsphere suspension was injected over 30 s after vigorous shaking to prevent ... ding of the spheres, and arterial blood was collected for a total of 4 min after injection of the spheres. ... procedure was repeated an hour later with differently labeled injectate.

After completion of arterial blood sampling, the test tube was disconnected, capped, centrifuged and ... alyzed for cerium and strontium radioactivity.

After two injections, the animal was killed with saturated KCl and the brain removed and divided into ... appropriate regions that were rapidly weighed in capped vials, homogenized in matched glass piston ... homogenizers and transferred to gamma-counting tubes.

... In preparation for manual intermittent sampling from the sagittal sinus, rats breathed ^{133}Xe for 15 min. The exposed dura was gently punctured with calibrated heat-sharpened glass capillary tube (Radiometer) and cerebral venous blood collected under gentle suction. A middle caudal arterial sample was ... taken simultaneously for determination of complete saturation of the animal with ^{133}Xe. The blood samples were immediately delivered under water to the bottom of standard fluorometer tube, stoppered and assayed for radioactivity.

Although care was taken to avoid leakage of cerebral venous blood around the puncture hole in the ... unstable arterial blood pressure nevertheless necessitated continuous infusion of fresh rat blood during the subsequent sampling period. After the initial sampling, the xenon gas mixture was disconnected and the animal ventilated with a xenon-free mixture of 70% N_2O and 30% O_2. During the subsequent ... desaturation phase, arterial and venous blood was sampled every minute for 10 min as described above.

Blood flow (ml 100 g^{-1} min^{-1}) in regions draining to the superior sagittal sinus was calculated from the following equation:

$$\mathrm{CBF} = 82\,\frac{c^{0}_{v} - c^{10}_{v}}{\int_{0}^{10}(c_v - c_a)\,dt}$$

Methods

Male Wistar rats, weighing 350–400 g and fed standard food pellets and water ad libitum, were [illegible] in 6 groups. Right and left cerebral hemisphere blood flow rates were measured in 15 rats (Group 1) [illegible] 15 μm [[illegible]Ce] microspheres. In 6 rats (Group 2) the blood flow rates of both hemispheres were [illegible] to the cerebellar and brainstem region blood flow rates, and in 6 rats (Group 3) the right hemisphere [illegible] flow was determined by successi e injection of 15 μm [[illegible] Ce] microspheres and 50 μm [[illegible]Sr] micro[illegible]

In 6 rats (Group 4) arteriovenous differences for o ygen concentration, oxygen tension, carbon [illegible] tension, and pH were determined from blood sampled from the tail artery and from the [illegible] sagittal sinus of the brain thro gh a burr hole in the sk ll 3 mm anterior to the torcular.

In 4 rats (Group 5) cortical blood flow and rate of oxygen consumption ere determined [illegible] desaturation as described by Eklof et al (1973) and Norbert and Siesjö (1974 a, 1974 b). In [illegible] (Group 6) a v differences for oxygen concentration, oxygen tension, carbon dioxide tension, [illegible] were measured by sampli g from the iliac rtery and from a catheter advanced to [illegible] 0-5 m[illegible] torcular via the transverse si us. In the same rats, whole-brain blood flow and metabolic rate [illegible] were measured during [133]Xe desaturation, as described by Gjedde et al (1975).

Surgical preparation

A) Preparation fo microsphere injection was ccomplished in 2 steps. On the day prior to C[illegible] [illegible]mination, rats were anesthetized with 4 ml divinyl ether administered in a 4 l jar. When the [illegible] longer responded to tilting of the jar they were quickly i jected with 0.3 mg kg⁻ b.wt. atropine intraperit neally and placed on thei backs with their heads in an ether-filled jar. A small [illegible] made in front of the anterio neck musculature, the right common carotid artery exposed, [illegible] incised a d a heparinized PP 50 (Portex) catheter advanced into the left ventricle of the heart. The [illegible] of the catheter in the ventricle was confirmed by blood pressure recording with a strain gauge [illegible] to a oscillograph. The distal end of the catheter was passed through a subcutaneous tunnel, [illegible] between the ears and f xed with a ligature. The distal end was capped, the incision closed with [illegible] animals allowed to recover until the next day The catheter was intermittently flushed [illegible]

The followi g day anesthesia was gain induced as described above, the animals ere [illegible] 3 mg kg⁻ b.wt. tubocurarine chloride i.p and artificially ventilated with 30% oxygen and 70 [illegible] oxide. A catheter (PP 50 (Portex)) was inserted into the t il artery for continuous monitoring [illegible] pressure with a strai gauge, nd for sampli g of arterial blood for determination of blood gas[illegible]

B) In preparation for measurement of [133]Xe desaturation by means of blood from the superior [illegible] sinus, rats were artificially entilated as described above, with 0.3 mCi 1⁻¹ [133]Xe added to the [illegible] gas mi ture administered from a 30 l Douglas bag A ta l artery catheter as inserted as [illegible] previously With dental drill, small hole was made in the superio sagittal suture of the skull [illegible] the anterio two thirds and the posterior third, care being taken not t rupture the dura [illegible] was sampled intermittently from the superior sagittal sinus and the tail artery for determ[illegible] cortical blood flow

C) In preparation f measurement of [133]Xe desaturation by means of blood from the confluens [illegible] rats were ventilated as described Incision sites inf ltrated with 0.5 lidocaine chloride, and placed in the tail artery the ilia artery via the femoral artery d the left transverse sinus [illegible] torcular as indicated by mark placed on 6 cm PX-010 (B & D) catheter 15 mm from the tip, [illegible] method of Gjedde t al (1975). Arterial blood was sampled from the ta l rtery for blood gas [illegible] pH determinations, while additional blood was sampled from the iliac artery and the transv[illegible] for determination of whole-brai blood flow and blood ga concentrations.

Determination f cerebral blood flow

Cerebral blood flow per 100 g of tissue (CBF) was calculated from the rate of brain tissue uptal[illegible] spheres) or release (xenon) of indicator:

$$\dot{Q} = CBF(c_a - c_v)$$

integrated to infinity:

$$CBF = \frac{Q}{\int^{\infty} |c_a - c_v| dt}$$

1 CBF of right and left hemispheres, cerebellum and brainstem regions in rats, measured with microspheres (experimental groups 1 2 & 3). All values ± S E number of observations.

Pa_{O_2}	Pa_{CO_2}	$_aPH$	Weight	^{141}Ce				^{85}Sr
mmHg			g	Right hem.	Left hem.	Cerebellum	Cranstem	Rt. hem.
09 6	37±1	7 34±0 02	346±14	191±79	218±30			
03±13	36±2	7.29±0 02	348± 7	201±35	223±34	152±19	158±30	
06 13	35 2	7 33±0 03	338± 6	236±34				214±29

II Arterial and venous gas values for cerebral cortex. Venous samples drawn from superior sagittal sinus. All values ± S E., number of observations.

Pa_{O_2}	Pa_{CO_2}	$_aPH$	$[O_2]_a$ ml l⁻¹	Pv_{O_2}	Pv_{O_2}	$_vPH$	$[O_2]_v$ ml l⁻¹
mmHg							
93 8	38_1	7 37±0 03	156±18	59±5	48±3	7 34±0 03	92±11

bellum and brainstem, measured with 15 μm [^{141}Ce] microspheres, and the blood flow of the right hemisphere, measured with 50 μm [^{85}S] microspheres, are tabulated able I. Size of microspheres did not affect the recorded flow rates, and no difference noted between the flow rates of right and left hemispheres. The flow rates of cerebellum brainstem ere about 25 % lower

Arterial and cerebral enous blood gas and pH values, recorded in 6 rats (Group 4), tabulated in Table II. Cerebral cortical blood flow and oxygen consumption rates of ats (Group 5) are tabulated in T ble III While blood gas and pH alues only required ple samples, multiple samples were needed to describe the complete washout curves of Xe from cortical tissue, and the two situations are therefore not directly comparable. a-v-[O₂]-deficit decreased with the increased number of samples, indicating departure in circulatory steady-state.

The arterial and enous gas and pH values, calculated from blood samples from the ansverse sinus near the torcular and the iliac artery are tabulated in Table IV Whole ain blood-flow and oxygen consumption rates determined by the same samples, are culated in Table V and Table IV and V are thus directly comparable The effect of fferent arterial carbon dioxide tensions on cerebral blood flow is apparent from Table V

TABLE III CBF and CMR_{O_2} of cerebral cortex, measured by means of ^{133}Xe desaturation from superior sagittal sinus. All alues ± S E., number of observations

Pa_{O_2}	Pa_{CO_2}	$_aPH$	CBF	CMR_{O_2}
mmHg			ml 100 g⁻¹ min⁻¹	
10_ 9	37+1	7 31_0 02	145 10	5 9_0 6

in which the constant 8 was composed of the tissue/blood partition coefficient for xenon (0 [...] *et al* 1975) and the standardized brain weight (100 g), c_v^s — superior sagittal sinus [133]Xe-con[...] complete saturation, c^{10} — superior sagittal sinus [133]Xe-concentration after 10 min desaturation [...] was calculated by the trapezoid rule (Norberg and Siesjö 1974 a) from the venous and arterial curves, c_v and c_a. Oxygen concentrations of separate early and late arterial and cerebral ve[...] obtained during desaturation were averaged for determination of cerebral oxygen consumption.

C) In preparation for constant rate sampling of cerebral venous blood from the confluens [...] breathed 0.3 mCi 1 [133]Xe for 24 min, at the end of which period av-saturation samples [...] 2 min from the transverse sinus and the iliac artery using a Harvard double-action pump [...] collect 100 μl min [133]Xe-administration was then terminated and the animal ventilated [...] mixture of 30–40% oxygen and 60–70% nitrous oxide. During the subsequent desaturation [...] desaturation samples were drawn for 4 min at 20 μl min⁻¹. Finally after desaturation [...] samples were collected for 2 min at 100 μl min.

Whole-brain blood flow (ml 100 g⁻ min) was calculated from the following equation

$$CBF = 10.15 \frac{T_X^{-4\ 0} - T_{v_X}^{24\ 26}}{T_{Xe}^{0\ 24} - T_{Xe}^{0\ 24}}$$

in which the constant 10.15 was composed of the tissue/blood partition coefficient for xe[...] brain of rat (1 015 Gjedde *et al* 1975), the standardized brain weight (100 g), and the [...] rates of blood sampling during, before and after the desaturation period, $T_{v_{Xe}}^{0\ 24}$ total r[...] cerebral venous sample drawn during desaturation, $T_{a_X}^{0\ 24}$ — total radioactivity of arterial [...] during desaturation, $T_X^{\ 2}$ — total radioactivity of cerebral venous sample at saturation, and [...] radioactivity of cerebral venous sample drawn after 4 min desaturation.

Autopsy always confirmed the intended position of the cerebral venous catheter

Blood and tissue samples

[14]C and [35]S radioactivities were determined by gamma-counting in an Auto-Gamma (Packard) at 135–155 keV and 500–520 keV discriminations. The individual radioactivi[...] labeled samples were calculated by the channels ratio method after inclusion of pure stron[...] [133]X radioactivity was determined at 50–145 keV discrimination (peak at 81 keV)

Cerebral rate of oxygen consumption (CMR_{O_2}) was calculated from the oxygen co[...] appropriate samples by the following equation

$$CMR_{O_2} = CBF(c_{aO_2} - c_{vO_2})$$

in which c_{aO_2} — mean arterial oxygen concentration during desaturation, and c_{vO_2} — mean c[...] oxygen concentration during desaturation. The oxygen concentrations (vol) of 20 μl sam[...] directly from the original specimens kept on ice were determined by carbon monoxide dep[...] Lex-O-Con (Lexington) charcoal fuel cell.

All animals were considered to be in respiratory steady-state when arterial carbon dioxi[...] tension determined at least 10 min apart agreed within 10%.

P_{O_2} and P_{CO_2} of whole blood were determined with Eschweiler microelectrodes, calibr[...] mixtures chosen to give minimal deviation from the expected values. pH in plasma was de[...] a Radiometer electrode and pH in whole blood was corrected by adding 0.01 pH and [...] compensate for the suspension effect (Severinghaus and Bradley 1969). All animals [...] temperature of 37 ± 0.3 C by a rectal thermoprobe connected to a thermostat and heat lam[...] was determined with Adams Auto-Crit centrifuge after 4 min centrifugation, and [...] sufficiently to influence calculations employing tissue/blood partition coefficients

Results

Cardiac output of one rat was 78 ml min or 189 ml kg min

Regional cerebral blood flow rates (Groups 1 2 & 3) of right and left h[...]

TABLE I. CBF of right and left hemispheres, cerebellum and brainstem regions in rats, measured with microspheres (experimental groups 1, 2 & 3). All values ± S.E. n = number of observations.

Pa_{O_2}	Pa_{CO_2}	apH	Weight	^{141}Ce				^{85}Sr
mmHg			g	Right hem.	Left hem.	Cerebellum	Cranstem	Rt. hem.
89 ± 6	37 ± 1	7.34 ± 0.02	344 ± 14	191 ± 29	18 ± 30			
93 ± 13	36 ± 2	7.29 ± 0.02	348 ± 7	201 ± 35	223 ± 34	15 ± 19	158 ± 30	
86 ± 13	35 +	7.33 ± 0.03	338 ± 6	256 ± 34				14 ± 29

TABLE II. Arterial and venous gas values for cerebral cortex. Venous samples drawn from superior sagittal sinus. All values ± S.E. n = number of observations.

Pa_{O_2}	Pa_{CO_2}	apH	$[O_2]_a$ ml l^{-1}	Pv_{O_2}	Pv_{CO_2}	vpH	$[O_2]_v$ ml l^{-1}
mmHg							
93 ± 6	34 ± 1	7.37 ± 0.03	156 ± 10	59 ± 5	48 ± 3	7.34 ± 0.03	97 ± 11

bellum and brainstem, measured with 15 µm [^{141}Ce] microspheres, and the blood flow of the right hemisphere, measured with 50 µm [^{85}Sr] microspheres, are tabulated in Table I. Size of microspheres did not affect the recorded flow rates, and no difference was noted between the flow rates of right and left hemispheres. The flow rates of cerebellum and brainstem were about 25% lower.

Arterial and cerebral venous blood gas and pH values, recorded in 6 rats (Group 4), are tabulated in Table II. Cerebral cortical blood flow and oxygen consumption rates of rats (Group 5) are tabulated in Table III. While blood gas and pH values only required single samples, multiple samples were needed to describe the complete washout curves of Xe from cortical tissue, and the two situations are therefore not directly comparable. The a-v-[O$_2$]-deficit decreased with the increased number of samples, indicating departure from circulatory steady-state.

The arterial and venous gas and pH values, calculated from blood samples from the transverse sinus near the torcular and the iliac artery, are tabulated in Table IV. Whole brain blood-flow and oxygen consumption rates determined by the same samples, are tabulated in Table V and Table IV and V are thus directly comparable. The effect of different arterial carbon dioxide tensions on cerebral blood flow is apparent from Table V.

TABLE III. CBF and CMR_{O_2} of cerebral cortex, measured by means of ^{133}Xe desaturation from superior sagittal sinus. All values ± S.E. n = number of observations.

Pa_{O_2}	Pa_{CO_2}	apH	CBF	CMR_{O_2}
mmHg			ml 100 g^{-1} min^{-1}	
107 ± 9	37 ± 1	7.31 ± 0.02	145 ± 10	5.9 ± 0.6

in which the constant 82 was composed of the tissue/blood partition coefficient for xenon (0.82, ... et / 1975) and the standardized brain weight (100 g), c_v^0 = superior sagittal sinus ^{133}Xe-concentration complete saturation, c^{10} = superior sagittal sinus ^{133}Xe-concentration after 10 min desaturation. The ... was calculated by the trapezoid rule (Norberg and Siesjö 1974 a) from the venous and arterial ... curves, c_v and c_a. Oxygen concentrations of separate early and late arterial and cerebral venous ... obtained during desaturation were averaged for determination of cerebral oxygen consumption ...

C) In preparation for constant rate sampling of cerebral venous blood from the confluens ... breathed 0.3 mCi 1 ^{133}Xe for 4 min at the end of which period av-saturation samples were drawn 2 min from the transverse sinus and the iliac artery using a Harvard double-action pump calibrated to collect 100 µl min^{-1}. ^{133}Xe-administration was then terminated and the animal ventilated with a mixture of 30–40% oxygen and 60–70% nitrous oxide. During the subsequent desaturation phase desaturation samples were drawn for 24 min at 20 µl min^{-1}. Finally after desaturation ... samples were collected for 2 min at 100 µl min

Whole-brain blood flow (ml 100 g^{-1} min^{-1}) was calculated from the following equation

$$CBF = 10.15 \frac{T_{Xe}^{-} - T_{vX}^{24}}{T_{X}^{0\ 24} - T_{Xe}^{0\ 24}}$$

in which the constant 10.15 was composed of the tissue/blood partition coefficient for xenon of the brain of rat (1.015, Gjedde et al. 1975), the standardized brain weight (100 g), and the different rates of blood sampling during, before and after the desaturation period, $T_{v_{Xe}}^{0\ 24}$ = total radioactivity of cerebral venous sample drawn during desaturation, $T_{Xe}^{0\ 24}$ = total radioactivity of arterial sample during desaturation, $T_{v_X}^{2}$ = total radioactivity of cerebral venous sample at saturation, and $T_{v_{Xe}}^{...}$ = radioactivity of cerebral venous sample drawn after 24 min desaturation.

Autopsy always confirmed the intended position of the cerebral venous catheter.

Blood and tissue analysis

Ce and ^{85}Sr radioactivities were determined by gamma-counting in ... Auto-Gamma Spectrometer (Packard) at 135–155 keV and 500–520 keV discriminations. The individual radioactivities of the labeled samples were calculated by the channels ratio method after inclusion of pure strontium ... ^{133}Xe radioactivity was determined at 50–125 keV discrimination (peak at 81 keV).

Cerebral rate of oxygen consumption (CMR$_{O_2}$) was calculated from the oxygen concentrations of appropriate samples by the following equation

$$CMR_{O_2} = CBF(c_{aO_2} - c_{vO_2})$$

in which c_{aO_2} = mean arterial oxygen concentration during desaturation, and c_{vO_2} = mean cerebral venous oxygen concentration during desaturation. The oxygen concentration (vol %) of 20 µl samples ... directly from the ... anaerobic specimens kept in ice were determined by carbon monoxide displacement ... Lex-O$_2$-Con (Lexington ...) charcoal fuel cell.

All animals were considered to be in respiratory steady-state when arterial carbon dioxide tension determined at least 10 min apart agreed within 10%.

P_{O_2} and P_{CO_2} of whole blood were determined with Eschweiler microelectrodes, calibrated with gas mixtures chosen to give minimal deviation from the expected values. pH of plasma was determined with a Radiometer electrode and pH in whole blood was corrected by adding 0.01 pH units ... compensate for the suspension effect (Severinghaus and Bradley 1969). All animals were kept at a temperature of 37±0.1 °C by a rectal thermoprobe connected to a thermostat and a heat lamp. Hematocrit was determined with an Adams Autocrit centrifuge after 4 min centrifugation, and never ... sufficiently to influence calculations employing tissue/blood partition coefficients.

Results

Cardiac output of one rat was 78 ml min^{-1} or 189 ml kg^{-1} min^{-1}.

Regional cerebral blood flow ...

… CBF of right and left hemispheres, cerebellum and brainstem regions in rats, measured with … microspheres (experimental groups 1 … & 3). All … alues ± S E., … number of observations.

…o₂	Paco₂	pH	Weight g	[14]Ce				[85]Sr
…mHg				Right hem	Left hem	Cerebellum	Brainstem	Rt. hem
…9 6	37 _ 1	7 34 _ 0 0…	346 ± 14	191 ± …9	18 _ 30			
…3 13	36 _	7.29 ± 0 02	348 _ 7	201 + 35	…3 + 34	15… _ 19	158 ± 30	
…6 ± 13	35 ± 2	7.33 ± 0 03	338 + 6	256 _ 34				214 _ …9

… II Arterial and …enous gas … alues for cerebral cortex. Venous samples dra… from superior sagittal sinus. All values ± S E … number of observations

P_{aO_2}	P_{aCO_2}	$_a$pH	$[O_2]_a$ ml l	P_{vO_2}	P_{vCO_2}	$_v$pH	$[O_2]_v$ ml l⁻¹
mmHg							
93 ± 8	38 1	7 37 + 0 03	156 18	59 5	48 3	7 34 ± 0 01	9 11

…bellum and brainstem, measured with 15 μm […Ce] microspheres, and the blood flow of the right hemisphere, measured with 50 μm [85Sr] microspheres, are tabulated …able I. Size of microspheres did not affect the recorded flow rates and no difference …noted between the flow rates of right and left hemispheres. The flow rates of cerebellum …brainstem were about 25 % lower

…rterial and cerebral venous blood gas and pH values, recorded in 6 rats (Group 4), …tabulated in Table II. Cerebral cortical blood flow and oxygen consumption rates of …ts (Group 5) are tabulated in Table III. While blood gas and pH …alues only required …le samples, multiple samples were needed to describe the complete washout curves of …Xe from cortical tissue, and the two situations are therefore not d rectly comparable …e av-$[O_2]$-deficit decreased with the increased number of samples, indicating departure …m circulatory steady-state.

The arterial and …enous gas and pH …alues, calculated from blood samples from the …nsverse sinus near the torcular and the iliac artery are tabulated in Table IV. Whole …ain blood-flow and oxygen consumption rates determined by the same samples, are …bulated in Table V and Table IV and V are thus directly comparable. The effect of …fferent arterial carbon dioxide tensions on cerebral blood flow is apparent from Table V

…ble III. CBF and CMR_{O_2} of cerebral cortex, measured by means of [133]X… desaturation from superior sagittal sinus. All …alues ± S E … number of observations

P_{aO_2}	P_{aCO_2}	$_a$pH	CBF	CMR_{O_2}
mmHg			ml 100 g⁻¹ min⁻¹	
102 9	37 _ 1	7 31 ± 0 02	143 ± 10	5 9 ± 0 6

TABLE IV Blood gas and pH values measured in arterial blood and in venous blood down transverse sinus within 0–5 mm of the torcular. All values ±S.E., n = number of ob

	Po_2	Pco_2	pH	$[O_2]$	n
	mmHg			ml l⁻¹	
Normocapnia					
Artery	117± 8	38±0.3	7.37±0.01	184±10	
Vein	45± 3	49±1.3	7.33±0.01	106± 4	10
Hypocapnia					
Artery	107±13	7±1.4	7.46±0.01	166± 9	
Vein	41± 2	37±1.7	7.41±0.01	7?± 8	10

Discussion

Independently of each other Sasaki and Wagner (1971) and Mendell and Hollenb published cerebral cardiac distribution indices for rat using microspheres. S Wagner allowed the animals 4 days recovery with the catheter in place in the le while Mendell and Hollenberg performed the CBF determinations immediately afte placement as did Pannier and Leusen (1973) who published cardiac distributi of rat brain during conditions of normal and abnormal acid base balance. The these three studies agree fairly well. Although microsphere size varied from 15 in the studies, and dextran glucose, or no suspension media were used to keep mic suspended, the cardiac distribution index of brain varied only from 1.5 to 2.6 brain. Pannier and Leusen also measured cardiac output and noted a value of min or approximately 170 ml kg min

In the present study cardiac output of one rat was similar viz. 189 ml kg m calculated cerebral blood flow rates were, however higher than those repo where and the animals, as a rule, were acidotic. Several explanations can be ad account for the high flow rates. The animals proved unable to recover completely surgery and trauma, and in addition may have been undernourished at the tim determination, as starvation ensues rapidly in the rat. Chronic acidosis was shown b and Leusen to be associated with an increase of cerebral blood flow at normoca the effect was enhanced if the animals hyperventilated prior to being ventilated a Neither Sasaki and Wagner nor Mendell and Hollenberg reported arterial pl Also, the cardiac outputs noted by Pannier and Leusen and in the present study w

TABLE V CBF and CMR_{O_2} of whole-brain, measured by means of ¹³³X desaturation usi g ve from the transverse sinus close t the torcula. All values ±S.E., n = number of ob

	Pa_{CO_2}	CBF	CMR_{O_2}	MABP	n
	mmHg	ml 100 g⁻¹ min⁻¹		mmHg	
Normocapnia	38±0.3	108±	8.0±0.6	1 7±5	10
Hypocapnia	27±1.4	60±5	6.1±0.7	117±6	10

... rial blood pressure. CO_2 – response = 4.1 ... per mmHg ... Paco₂

m the also reported by Sapirstein and Hanusek (1958) and suggest a direct effect of heterization on the circulatory condition of the animals.

The results obtained her indicated no significant differences between cerebral blood flow values measured with spheres of 15 and of 50 μm diameter and no significant difference between the flow values measured in the two hemispheres, despite ligation of the right common carotid artery. The flow rates measured in the cerebellum and lower brain regions were lower than those of the hemispheres but in all three groups standard errors were large, indicating pathological variation of the condition of the animals used in the study.

The "non-invasive" indicator exchange method as originally described by Kety and Schmidt (1945) was first used in rat by Eklöf et al. (1973) and Norberg and Siesjö (1974 a, 74 b) who measured cerebral cortical blood flow and oxygen consumption rates by sampling venous blood intermittently from the superior sagittal sinus during ^{133}Xe desaturation. At normocapnia, cortical blood flow was 100 ml 100 g^{-1} min and cortical oxygen consumption 10 ml 100 g^{-1} min. With this method, CBF varied from 64 to 465 ml 100 g^{-1} min^{-1} when Pa_{CO_2} varied from 13 to 89 mmHg (Eklöf et al. (1973)). Thus the cortical blood flow rates were higher than reported for other species by the same technique, and the av-[O_2]-deficits larger than reported by others for rat at the same Pa_{CO_2}-tension. Although Hawkins et al. (1973) reported similar values for cortical blood flow (100 ml 100 g^{-1} min^{-1}) and oxygen consumption (10.4 ml 100 g^{-} min), CBF was calculated directly from av-deficits and cerebral metabolic rate, and oxygen consumption exceeded glucose consumption by 20 In brain, allegedly because of ketonemia.

In the present study sampling from the superior sagittal sinus of the rat, single av-samples yielded av-[O_2]-deficits close to 64 ml l at normocapnia. When multiple samples were taken for simultaneous determination of cerebral blood flow and oxygen consumption, however the [O_2]-deficit decreased significantly during the period of sampling (and desaturation), the mean for the late samples being 40 ml l. Thus the constant flow required for the CBF-determinations may not have existed. Therefore the CMR_{O_2}-value 4 ml 100 g^{-} min) obtained from the calculated CBF and the mean [O_2]-deficit should be regarded with considerable reserve.

Nilsson (1974), approaching the rat's cerebral circulation via the internal maxillary vein, described desaturation curves for ^{133}Xe and, compressing the contralateral outflow channel at the level of the postglenoid foramen, obtained an av-[O_2]-deficit of 95 ml l^{-} t normocapnia. Compression of the lateral outflow tract may however have caused redirection of blood of different origin. With compression, CBF was 80 ml 100 g^{-} min without 50 ml 100 g^{-} min with an av-[O_2]-deficit of 70 ml l. The decrease was attributed to extracerebral contamination although 70 ml l^{-} seems closer to the av-[O_2]-deficit of most brain reports reported by others at normocapnia.

Nilsson and Siesjö (1976) reported CBF decrease of 1.5% in hypocapnia, and a CBF increase of 8.6 in hypercapnia, per mmHg change of normocapnic Pa_{CO_2}. The inhomogeneity of this CO_2-response exceeds previously reported observations.

Using Nilsson's approach to the transverse sinus of the rat brain, Gjedde et al. (1975) reported whole-brain blood flow of 98 ml 100 g^{-1} min^{-1} and a CMR_{O_2} of 5.4 ml 100 g^{-1} min^{-1} when blood was sampled from the distal part of the transverse sinus. Extra-

TABLE IV. Blood gas and pH-values measured in arterial blood and in venous blood drawn from transverse sinus within 0–5 mm of the torcular. All values ±S.E., n = number of observations

	P_{O_2}	P_{CO_2}	pH	$[O_2]$	n
	mmHg			ml l^{-1}	
Normocapnia					
Artery	117 ± 8	38 ± 0.3	7.37 ± 0.01	184 ± 10	
Vein	45 ± 3	49 ± 1.3	7.33 ± 0.01	106 ± 4	10
Hypocapnia					
Artery	107 ± 13	27 ± 1.4	7.46 ± 0.01	166 ± 9	
Vein	41 ± 2	37 ± 1.7	7.41 ± 0.01	72 ± 8	10

Discussion

Independently of each other Sasaki and Wagner (1971) and Mendell and Hollenberg (1971) published cerebral cardiac distribution indices for rat, using microspheres. Sasaki and Wagner allowed the animals 4 days recovery with the catheter in place in the left ventricle while Mendell and Hollenberg performed the CBF determinations immediately after catheter placement, as did Pannier and Leusen (1973) who published cardiac distribution indices of rat brain during conditions of normal and abnormal acid–base balance. The results of these three studies agree fairly well. Although microsphere size varied from 15 to 50 in the studies, and dextran, glucose, or no suspension media were used to keep microspheres suspended, the cardiac distribution index of brain varied only from 1.5 to 2.6 per ... brain. Pannier and Leusen also measured cardiac output and noted a value of 51 ... min or approximately 170 ml kg⁻ min

In the present study cardiac output of one rat was similar *viz* 189 ml kg min ... calculated cerebral blood flow rates were, however, higher than those reported elsewhere and the animals, as a rule, were acidotic. Several explanations can be advanced to account for the high flow rates. The animals proved unable to recover completely from surgery and trauma, and, in addition, may have been undernourished at the time of determination, as starvation ensues rapidly in the rat. Chronic acidosis was shown by Pannier and Leusen to be associated with an increase of cerebral blood flow at normocapnia, the effect was enhanced if the animals hyperventilated prior to being ventilated artificially. Neither Sasaki and Wagner nor Mendell and Hollenberg reported arterial pH values. Also the cardiac outputs noted by Pannier and Leusen and in the present study were ...

TABLE V. CBF and CMR_{O_2} of whole brain, measured by means of ^{133}Xe desaturation using ... from the transverse sinus close to the torcula. All values ±S.E., n = number of observations

	Pa_{CO_2}	CBF	CMR_{O_2}	MABP	n
	mmHg	ml 100 g⁻¹ min⁻¹		mmHg	
Normocapnia	38 ± 0.3	108 ± 2	8.0 ± 0.6	127 ± 5	10
Hypocapnia	27 ± 1.4	60 ± 5	6.1 ± 0.7	117 ± 6	10

MABP = mean arterial blood pressure. CO_2-response = 4.1 ... per mmHg change of Pa_{CO_2}.

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cerebral contamination was judged to be minimal However the av-[O_2]-deficit was ... 56 ml l and CBF varied from 42 to 181 ml 100 g^{-1} min when Pa_{CO_2} varied from ... 75 mmHg.

In the present study using the same approach but advancing the catheter to a posi... close to the torcular the av [O_2]-deficit was higher (78 ml l) although cerebral blood fl... was similar Hawkins & Veech (1974) sampled cerebral venous blood directly from t... torcular and obtained an av [O_2]-deficit of 64 ml l and an av-[glucose]-deficit of ... μmol l^{-1} Assuming a torcular blood flow of at the most 100 ml 100 g^{-1} min^{-1} th... values correspond to a CMR_{O_2} of 6 4 ml 100 g^{-} min *i.e* somewhat less than observed in ... present study

In conclusion microspheres offer certain disadvantages when applied to the measure... ment of cerebral blood flow in small laboratory animals Their administration requires lig... tion of one carotid artery a procedure which, although safe in normal animals (Le... 1960) may introduce errors in animals in which catheter placement may alter card... output. Thus, the presence of a relatively large bore catheter in the aortic valve closure ... may have contributed to the high cerebral blood flow rates observed with this metho...

While the use of inert indicator exchange offers the advantage of simultaneous deter... minations of cerebral blood flow and oxygen consumption rates, manual intermitt... sampling from the superior sagittal sinus may impede the maintenance of circulato... steady-state demanded by the method

On the other hand continuous sampling appears to be less traumatic to the animal ... therefore impose less strain on the circulation while minor changes in CBF and ve... oxygen concentration are of opposite directions and thus tend to produce results that ... less divergent than the individual CBF or oxygen concentration variations.

Although disagreement exists as to the most accurate estimate of whole-brain oxy... consumption rates in the rat, and to the topography of 'whole brain blood flow' of ... species, including man primate and dog, share whole brain av-[O]-deficits of 60-70 ml l ... differences in metabolic demand being met by differences in blood flow rates. The de... mination of regional glucose consumption rates in the rat brain by the 2-deoxy-D-gluc... method confirms that in several species the metabolic rate of cortex is much greater th... that of the brain as a whole (Plum *et al* 1976) Although the exact rate of cort... metabolism in the rat remains in dispute whole-brain oxygen consumption rate appe... to be close to 7 ml 100 g min

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The synaptic effect of catecholamines is assumed to be mediated by activation of adenylate ... located to postsynaptic structures (Bloom 1975). Thus dopamine and norepinephrine ... ate adenylate cyclase in the rat striatum (Kebabian *et al.* 1972).

... epletion of dopamine in rat striatum by unilateral 6-hydroxydopamine administration ... substantia nigra and radio frequency lesions of this area induced denervation hyper... itivity of the striatal dopamine receptor as evidenced by rotational behaviour and en... ced adenylate cyclase activity in striatum (Mishra *et al.* 1974).

... epletion of brain catecholamines in experimental hepatic failure might induce hyper... itivity of the dopamine and norepinephrine receptors and associated adenylate cyclases. ...refore, ...e have studied dopamine-sensitive and norepinephrine-sensitive adenylate ...ases in striatum from rats with porto-caval anastomosis.

Material and methods

Wistar rats initially weighing 200–310 g and 16–20 weeks old were used. The animals were housed ... cages and fed *ad libitum* on ... commercial diet (Rostock, Korn- og Foderstofkompagniet, Copen...n, Denmark).

... end to-side porto-caval anastomosis (Lee and Fisher 1961) ... as established in 14 animals in ether ...thesia. A non-sterile, but clean, surgical technique was used. The abdomen ... as opened ... ith an in... in the linea alba and the intestine was retracted to the left to expose the inferior vena cava and portal ... The coronary ... was ligated and cut off. Subsequently the portal vein was clamped distally and ... enters the liver, and cut. The mobilized vein ... as attached ... ith 7-0 silk to an incision in the antero... ...ral wall of the inferior vena cava just above the right renal vein. Continuous sutures were used. The ...als recovered ... ithin ... few minutes after the operation. The patency of the anastomosis was ...erified ...ry case after sacrifice.

... sham operation was carried out on 13 rats. The surgical procedure ... as identical to the one described ..., except that no anastomosis ... as performed. However, the portal vein and the inferior vena cava ... clamped for 12 min—the time used to establish the anastomosis in the shunted rats.

...Four weeks after the operations the adenylate cyclase activity ... as measured.

...Adenylate cyclase assay. The method used ... as essentially as described by Kebabian *et al.* (1972). The ... were decapitated in ... cold room (4°C).

The brain stem and cerebellum were removed, and the brain ... as hemisected along the midline. The ... ral ventricle of the cerebral hemispheres was opened ... ith an incision inferior to the corpus callosum ... caudate putamen (Zeman and Innes 1963) was separated on both sides from the internal capsule, ...ed, and placed in ... cold Krebs–Ringer bicarbonate buffer (122 mM NaCl, 3 mM KCl, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, 0.4 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM d-glucose, pH 7.4). The tissue ... as homo...nized by hand in 25 vol. of an ice-cold 2 mM Tris-maleate buffer pH 7.4 containing 2 mM EGTA. ... µl of the homogenate were preincubated for 30 min at 0°C in 400 µl medium containing 100 mM Tris, ...0 mM maleate, 12 mM theophylline, 2.5 mM $MgSO_4$, 0.25 mM EGTA, and hormones in concentrations ...icated in the results. After preincubation the reactions ... ere started by adding 50 µl of ... 5 mM ATP ...ution giving ... final volume of 500 µl. The incubates were maintained at 30°C in ... shaking ... ater bath for 5 min. The reactions ... ere terminated by boiling for 3 min. The incubates were centrifuged at 2 800 G ... 10 min, and the supernatants were frozen and stored at −20°C until determination of cyclic AMP. ...yclic AMP was measured by ... method previously described (Gerstler *et al.* 1976).

In short, cyclic AMP ... as determined by ... competitive protein binding method using ... binding protein ...ially prepared as described by Miyamoto *et al.* (1969).

Standards were prepared by mixing 50 µl of solutions of know... cyclic AMP concentrations and 50 µl ...f blank tissue preparation, while samples contained 50 µl of buffer and 50 µl of incubated ...ssue. 50 µl ...f binding protein and 50 µl of tritiated cyclic AMP ... ere added to all tubes. After mixing the contents, ...hey were incubated at 4°C for 90 min. Incubation was terminated by adding 1 ml of ... 70 ... ammonium ...lphate solution containing 10 mg calcium sulphate. After 10 min the tubes were centrifuged for 20 min ... 2 800 ... G and 4°C. Next the supernatants were decanted and the precipitates were redissolved by adding ...0 µl of water and 1.2 ml Insta-gel. After mixing, the tubes ... ere placed in scintillation counting vials and ...nted to ... minimum of ten thousand counts.

Acta physiol. scand. 1977 100 282 287

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Adenylate Cyclase Activity in Corpus Striatum of Rats with Porto-Caval Anastomosis

By

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Received 22 September 1976

Abstract

LAURSEN H., R. KLYSNER and A GEISLER. *Adenylate cyclase activity in corpus striatum of rats with porto-caval anastomosis* Acta physiol. scand. 1977 *100* 282 287

Altered catecholamine receptor sites within the striatum have been proposed to be an important pathogenetic factor in hepatic and porto-systemic encephalopathy and coma. The unstimulated, fluoride-, norepinephrine and dopamine-stimulated adenylate cyclase activity were measured in the corpus striatum of rats with a four weeks old end-to-side porto-caval anastomosis. There was no difference in unstimulated, fluoride- or hormone-stimulated adenylate cyclase activity between porto-caval shunted and sham-operated rats. The *in vitro* dose-response curves of norepinephrine and dopamine were similar in both groups of animals. Half maximum and maximum stimulation were achieved in shunted and sham-operated at identical concentrations of norepinephrine and dopamine, respectively The results indicate that no changes in unstimulated adenylate cyclase activity nor changes in the response of adenylate cyclase activity to fluoride, norepinephrine and dopamine had developed in the rats at the stage studied.

Key words. Porto-caval anastomosis, encephalopathy corpus striatum, adenylate cyclase

Hepatic (Adams and Foley 1953 Victor *et al* 1965) and porto-systemic encephalopathy (Sherlock *et al* 1954) include neurological (choreoathetosis, rigidity and asterixis) and mental symptoms. These are aggravated by ingestion of phenylalanine (Condon 19..) while L-dopa (L 3 4-dihydroxyphenylalanine)—a precursor of dopamine and norepinephrine —has an ameliorating effect (Fischer and James 1972) The synthesis of L-dopa from tyrosine by tyrosine hydroxylase is rate-limiting in the formation of catecholamines. However phenylalanine is also a substrate for tyrosine hydroxylase, resulting in formation of 3-hydroxyphenylethylamines (Curtius *et al* 1972) and it may inhibit tyrosine hydroxylase leading to an increased decarboxylation of tyrosine to tyramine and subsequently to octopamine by beta-hydroxylation (Brissot and Bourel 1974).

Plasma phenylalanine is elevated in patients with liver diseases (Iob *et al.* 1966) and in rats with experimental hepatic failure (Fischer 1974) This may explain the decreased level of norepinephrine and increased level of octopamine in brains from rats with experimental hepatic failure observed by Fischer (1974).

I Effect of dopamine, norepinephrine and sodium fluoride on adenylate cyclase activity of the striatum of rats with porto-caval anastomosis and sham-operated rats. The results are means ±S E M, n number of animals

	Adenylate cyclase activity[a] Porto-caval anastomosis		Sham-operated	
...ulated	11 6±0 95	14	10 9±0 7?	13
...mine 500 µM	?4 6±2 9?	8	.3 7 ...4	7
...nephrine 500 µM	.3 0_ 96	8	.2 6±1 9?	7
...ride 10 mM	23.3_2.15	8	24 7±1.?7	7

...ayed in duplicates in all animals and expressed as pmoles of cyclic AMP formed per 2 mg of striatal ... per 3 min

two groups of animals. Maximum stimulation of adenylate cyclase activity amounting ...00% of unstimulated activity was induced by dopamine in a concentration of 1 000 µM ...f-maximum activation was achieved by a dopamine concentration of 10 µM

...n other experiments the unstimulated adenylate cyclase activity in homogenates from ...m-operated rats did not differ from that of rats with porto-caval anastomosis (Table I). ...thermore, activation of adenylate cyclase with 10 mM sodium fluoride, a non-specific ...ivator of adenylate cyclase in homogenates from various tissues including brain tissue, ...uced an increase of enzyme activity to the same degree in both groups of animals. Simil...y no differences were observed between anastomosed and sham-operated rats when ...nylate cyclase in striatal homogenates was stimulated by nearly maximum (500 µM) ...centrations of dopamine and norepinephrine. The activation achieved by addition of ...ium fluoride was comparable to that obtained by addition of dopamine and norepine...rine.

Discussion

...e neurological symptoms in porto-systemic and hepatic encephalopathy may be caused by ...tal dysfunctions. This is supported by the finding of enhanced numbers of Alzheimer ...e II astrocytes in striatum of patients with these diseases (Adams and Foley 1953). Rats ...th porto-ca al anastomosis have morphological astrocyte changes (Laursen *et al* 1975) ...d a decrease in the number of neurones (Diemer *et al* 1976)

It has been suggested that the striatal lesions observed in porto-systemic encephalopathy ...y impair synaptic transmission and induce an alteration in catecholamine receptor sites ...isans and Weiner 1976). The dopamine-stimulated adenylate cyclase in striatum has ...en proposed to be identical to "the dopamine receptor" (Kebabian *et al* 1972). Enhanced ...ponsiveness of this enzyme has been reported after treatments which are known to induce ...persensitivity of dopamine receptors by depletion of dopamine (Mishra *et al* 1974) and ...blockade of dopamine receptors by haloperidol (Iwatsubo and Clouet 1975), a drug with ...rkinsonian side effects.

In this study of rats with porto-caval anastomosis, no changes in unstimulated and ...techolamine-stimulated adenylate cyclase activity could be observed in striatal homo...ates. The unstimulated and fluoride-stimulated adenylate cyclase activity were identical

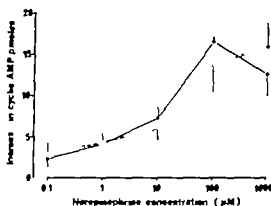


Fig. 1 Dose-response curves for norepinephrine cyclic AMP production in striatal homogenates rats with a porto-caval anastomosis (● ●) r sham-operated rats (O— O). Increase in cyclic AMP refers to the increase in cyclic AMP production during 2.5 min incubation over control value absence of added drug. Control values for experiments from rats with porto-caval anastomosis 11 0±1.2 pmoles cyclic AMP formed per 2 tissue per 2.5 min and 11.6±0.9 for sham-operated rats. Each point is the mean ±S.E.M for 6 determinations. The data were evaluated by x^2-test

Results

The general condition of the animals remained good after the operations, but the sham rats tended to become unkempt and infested with lice No disturbance in motor activity was observed A transient decrease in b wt of about 10 % occurred in shunted animals during the first week following the operation Afterwards, the rats gained weight normally There was no weight loss in the sham-operated rats. There was no difference in b.wt. between the two groups of rats when the adenylate cyclase activity was estimated (sham-operated 33 g, porto-caval anastomosis 328 ± 18 g, mean ± 2 S E.M) The inspection of the anastomosis verified that it was intact in all the operated animals.

The norepinephrine dopamine fluoride and unstimulated adenylate cyclase activity were estimated When norepinephrine in concentrations from 0.1 μM to 1 000 μM was added to striatal homogenates from rats with porto-caval anastomosis and sham-operated rats during the preincubation period of 30 min dose-dependent increases in cyclic AMP formation were observed (Fig 1) Maximum stimulation of the adenylate cyclase activity amounting to 250% of unstimulated activity was achieved by norepinephrine in a concentration of 1 000 μM in both groups of rats. Half maximum stimulations of adenylate cyclase were produced by 20 μM of norepinephrine in anastomosed and sham-operated rats

Similarly dopamine in concentrations from 0 1 μM to 1 000 μM activated adenylate cyclase in a dose-dependent manner (Fig. 2) The dose-response curves were identical f

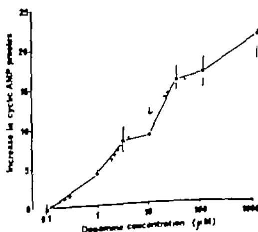


Fig. 2. Dose-response curves for dopamine on cyclic AMP production in striatal homogenates from rats with porto-caval anastomosis (●—●) and sham-operated rat (O -O). Increase in cyclic AMP refers to the increase in cyclic AMP production during 2.5 min incubation over control values in absence of added drug. Control values for experiments from rats with porto-caval anastomosis were 11.0 pmoles cyclic AMP formed per mg tissue per 2.5 min and 11 6±0 9 for sham-operated rats. Each point the mean ±S.E.M for 6 determinations. The data were evaluated by x^2 test

I Effect of dopamine, norepinephrine and sodium fluoride on adenylate cyclase activity of the striatum in rats with porto-caval anastomosis and sham-operated rats. The results are means ±S.E.M. number of animals.

	Adenylate cyclase activity Porto-caval anastomosis		Sham-operated	
[illegible]ulated	11 6±0 95	14	10 9±0 72	13
[illegible]mine 500 μM	24 6±2 98	8	25 7± 24	7
[illegible]nephrine 500 μM	23 0±2 96	8	22 6±1.98	7
[illegible]ride 10 mM	23.3±2 15	8	4 7±1 87	7

[illegible]ayed in duplicates in all animals and expressed as pmoles of cyclic AMP formed per 2 mg of striatal per 2 5 min

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in both groups. These results indicate that the amount of adenylate cyclase in the was unaffected in rats with porto-caval anastomosis. Furthermore, the dose-respons for dopamine and norepinephrine did not differ in the two groups, indicating that no in receptor sensitivity or total receptor activity had developed four weeks after the op

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In patients with hepatic encephalopathy urinary excretion and serum levels o pamine were increased (Lam *et al* 1973 Manghani *et al* 1975), but the origin of oct in serum is unknown However a defect in cerebral catecholamine metabolism is su by the striking, though temporary improvement of the level of consciousness and E normalities obtained in patients in hepatic coma, when treated with L-dopa (Park 1970)

The present results do not support the assumption of a deranged function of catecholamine synapses in porto-systemic encephalopathy but it cannot be exclud this may occur in the more severe cerebral disturbances during hepatic coma.

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[illegible] Individual and mean values ± S.E. for age, height, weight, pulmonary oxygen uptake during exercise, and work loads for the arms and for the legs.

	JO	CN	HT	EM	LE	PT	JM	Mean	S.E.
[illegible]	22	22	24	21	26	25	23	23.3	0.68
[illegible], cm	193	181	192	192	180	189	178	186	2.4
[illegible], kg	71	72	104	84	87	74	72	81	4.6
[illegible], l min⁻¹ STPD									
[illegible]	2.78	3.39	3.99	3.23	4.02	3.00	2.92	3.3	0.17
[illegible]	3.38	3.40	5.69	4.14	4.61	3.60	3.37	4.0	0.33
[illegible]	3.72	3.57	5.98	4.98	5.45	3.71	3.87	4.4	0.36
[illegible] min⁻¹ STPD									
[illegible]	1.09	1.29	[illegible].19	1.39	1.67	1.22	1.26	1.4	0.14
[illegible]	2.63	2.59	3.31	2.59	2.89	2.29	2.40	2.7	0.13
[illegible] after arm									
[illegible]	3.04	3.09	4.65	3.50	3.83	2.85	3.13	3.4	0.24
[illegible] after leg									
[illegible]	3.14	2.85	4.6	3.54	3.71	2.91	3.13	3.4	0.23
percent of V_{O_2} max									
[illegible]	40.2	38.3	62.1	42.9	41.6	40.8	41.9	44	3.1
[illegible]	77.9	76.0	58.1	62.6	62.6	63.5	71.2	67	2.9
[illegible]	83.1	83.2	77.3	70.7	71.8	77.6	80.9	78	1.9
load, Watt									
[illegible]	80	65	100	65	75	55	60	69	5.7
[illegible]	160	155	250	200	230	170	180	190	13
[illegible]	Athletics	Athletics	Rowing	Soccer	Rowing	Handball	Basketball		
[illegible]	▲	▼	●	■	◀	▶	◆		

Methods and Procedures

[illegible] young males were studied after their informed consent was obtained. Age, height, weight and [illegible] data are given in Table 1. All [illegible] above average state of physical training and familiar [illegible] dynamic exercise through participation in various sport activities. Before the main study [illegible] catheterization, each subject came to the laboratory at 4 different days for determination of [illegible] during leg exercise, arm exercise and combined arm and leg exercise (combined exercise) and for [illegible] of appropriate submaximal work loads to be used in the main study. The procedure used [illegible] V_{O_2} max determination [illegible] described elsewhere (Secher et al. 1974).

The main study took place in the morning. The subjects had been allowed to eat [illegible] light meal 2–3 h [illegible]. Three catheters were inserted percutaneously so as to allow sampling of blood from the right [illegible] artery and vein at the level of the sacroiliac joint and from the right axillary vein at the level [illegible] the coracoid processus of the scapula.

After 30 min of rest the following measurements were made with the subject still lying on the catheterization table. 1. Collection of expired air for pulmonary ventilation ($\dot{V}$), oxygen uptake (V_{O_2}) and carbon [illegible] output (V_{CO_2}). 2. Measurement of cardiac output ($\dot{Q}$) and sampling of blood from all three [illegible] in duplicate to be analyzed for O_2 saturation, hemoglobin and lactate concentrations. 3. [illegible] of ECG and arterial blood pressure.

T[illegible] Elema-Schönander electrically-braked constant load, variable speed ergometers were used for the [illegible] exercise tests. The subjects were seated in the upright position, the pedals of the leg exercise [illegible] being almost vertically under the saddle. The ergometer used for arm exercise was placed in [illegible] of the subject, so that cranking [illegible] performed with the arms at the horizontal position. Each subject [illegible] two 20 min exercise periods. The protocol appears from Fig. 1 and 2. During the first 10 min [illegible] the first period the subject exercised with the legs alone (3 subjects) or with the arms alone (4 subjects) [illegible] thereafter arm exercise (respectively leg exercise) was superimposed on the ongoing exercise, and combined exercise was continued for the remaining 10 min. In each subject the work loads were chosen [illegible] to lead to complete exhaustion at the end of each 20 min exercise period. As shown in Fig. 1 during

Acta physiol. scand. 1977 100 288 297

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Central and Regional Circulatory Effects of Adding Arm Exercise to Leg Exercise

By

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Received 4 October 1976

Abstract

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regional circulatory effects of adding arm exercise to leg exercise Acta phys
1977 *100* 288–297

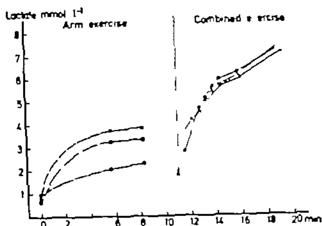
7 young, healthy male subjects performed exercise on bicycle ergometers in tw 20 mi peri interval of 1 h. The f rst 10 m of each 20 min period consisted of rm exercise (38–6_ of V_{O_2} exercise) or leg exercise (58–78 % of V_{O_2} ma for leg exercise) During the last 10 min the formed combined arm and leg exercise (71–83 of Vo max for this type of exercise) Th variables were measured dur g each type of exercise o ygen uptake heart rate mean ar pressure, cardia output, leg blood flow (only d ring leg xercise and combined exercise), ar concentration differences for O_2 and lactate t the levels of the axillary and the external ilia

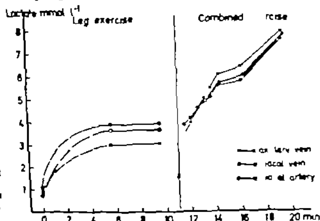
S perimposi g a sufficiently strenuous rm exercise (xygen ptake f r arm exercise 40 uptak f combined exercise) on leg exercise caused reduction n blood flow and oxygen u exerci g legs w th unchanged mean arterial blood pressure. S perimposing leg exercise on a caused decrease n mean rterial blood pressure d an increased a illary rteno- ex d fference. These f nd gs ndicate that the o ygen supply t one large group of exercising m be l mited by vasoconstriction by f ll i rterial pressure when another large group of exercising simultaneously

Key ord Arm exercise leg exercise combined rm nd leg e ercise cardiac utput leg b mea arterial blood pressure t tal nd regional ascul resista nce factors limit ng oxygen

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The maximal pulmonary oxygen uptake (V_{O_2} max) during leg exercise is only increased by addition of arm exercise (Taylor *et al* 1955 Åstrand and Saltin 1961 *et al* 1967 Kamon and Pandolf 1972, Hermansen 1973 Secher *et al* 1974 Se Oddershede 1975) This might reflect that exercise with one muscle group in oxygen uptake in another acti e muscle group In order to investigate this problem the effects on blood flow and oxygen uptake in the legs of superimposing arm ex leg exercise or leg exercise on arm exercise were studied To evaluate concomitant m changes arterial and regional venous blood concentrations of lactate were m





2. Group average blood lac concentrations plotted against during the two 20 min exer periods. Broken lines drawn ording to Klausen et al. (1974)

moglobin concentration were determined spectrophotometrically (Holmgren and Pernow 1959). Blood ygen concentration was calculated assuming that 1 g of hemoglobin combines with 1.39 ml of oxygen as pure oxygen is used for calibration (Gregory et al. 1971). Blood lactate was analyzed by an enzymatic thod (Scholz et al. 1959). HR was counted from the continuous ECG recordings. Intra-arterial blood ssure as measured as previously described (Clausen et al. 1969). Mean arterial blood pressure (MBP) calculated as the diastolic pressure plus 1/3 of the pulse pressure. Total vascular resistance (TVR) and leg vascular resistance (LVR) ere calculated by dividing MBP by $\dot{Q}$ and LBF respectively. The culation of LVR was done assuming that the influence on blood flow of muscle contractions is the me at given work load and rate of revolutions.

stics

andard deviations (S.D.) and standard errors (S.E.) ere calculated and differences were analyzed by the udent t-test for paired observations.

Results

dividual values and mean values for the work loads and for maximal and submaximal $\dot{V}O_2$, during the three types of exercise are listed in Table I. On an average 36% (range 30–42) of the total work output during combined exercise was performed with the arms. Expressed

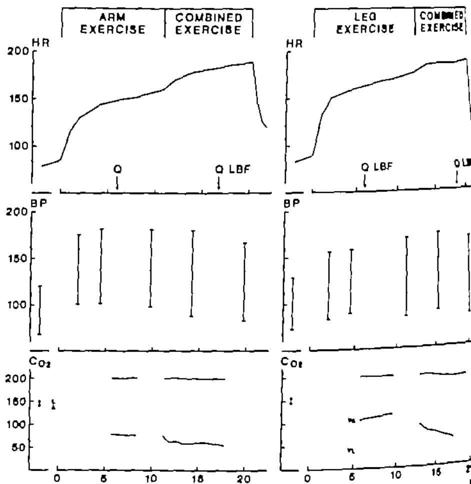


Fig. 1 Group average values for heart rate (HR), beats min^{-1} arterial blood pressure (BP), and blood oxygen concentration (C_{O_2}) ml l^{-1} plotted against time during the two 20 min exercise Times f r determination of cardia output (Q̇) and leg blood flow (LBF) are indicated by a = arterial, VA = axillary vein, VL = il ac vein

each type of exercise Q̇ was determined after about 7 min of exercise. During leg exercise and exercise the Q̇ determinatio was f llowed by measurement of the leg blood flow (LBF). Blood to be analyzed f O_2 saturation, hemoglobi and lactate concentrations were drawn from catheters immediately before od after the measurements of Q̇ and LBF In dd tion rterial blood samples to be analyzed for O_2 saturation and lactate concentrations were drawn at short duri g the first 3 mi starting at the transition from leg exercise (arm exercise) to combined During each type of exercise expired ai was collected from the sixth to approximately the ECG in one precordial lead was recorded throughout the exercise periods and arterial blood pre monitored continuously interrupted by the sampling of arterial blood and measurement of Q̇ only

The second 20 min exercise period was performed after 60 mi of supine rest. The protoco work loads were the same as the first period, except that the subjects who had started th leg the first period started with arm exercise in the second and vice versa

The determinations of V_E, V_{O_2} and $\dot{V}_{CO_2}$ were made using the Do glas bag method Q̇ and measured by single injection indicator d luti n techniques sing ^{99m}T pertechnetat as a tracer ^{131}I labelled human albumin as tracer for LBF The coefficient of variation for double deter in the present subjects during exercise was 4.0 % f r Q̇ and 4 4 % for LBF LBF measured in on the f llowing multiplied by two t represent blood flow i both legs. Blood oxygen saturation

...ll Regional, circulatory and metabolic data at rest and during three types of exercise. LBF LVR and Leg $\dot{V}o_2$ given as values for both legs.

	LBF l min⁻¹	LVR mmHg min l⁻¹	(a-v)O₂diff., ml l⁻¹ Legs	Arms	Leg $\dot{V}o_2$ l min⁻¹	Blood lactate concentration, mmol l⁻¹ a.	fem.	axil.
[illegible]	—	—	31	26	—	0.79	0.90	0.82
	—	—	2.9	1 7	—	0.95	0 06	0 07
[illegible]	—	—	108	1 4	—	3.3	[illegible]3	3.8
	—	—	8 4	3 7	—	0 19	0.16	0.18
[illegible]	10 8	10 9	178	142	1.9	7.0	7	7.2
	0.71	1.0	4 6	6	0 15	0.48	0 50	0 36
[illegible]	—	—	70	18	—	3 7	4 9	3 4
[illegible]	—	—	8 7	5.1	—	0 54	0 55	0 40
[illegible]	—	—	7 98	3 51	—	6.86	8.80	8 42
[illegible]	12 4	9 1	156	89	2 0	3 7	3 9	3 6
	0 95	0.73	5.7	10 5	0 20	0 63	0 6[illegible]	0.36
[illegible]	10 5	10 9	176	136	1.8	7 7	7 5	7 9
E.	0 63	0 96	4.5	7[illegible]	0.12	0 85	0 89	0.76
[illegible]	1 9	1.8	20	47	0 2	4 0	3 6	4 9
E.	0 77	0 51	4.5	7 5	0.14	0 51	0 55	0 57
[illegible]	2 75	3.48	4 [illegible]5	6 22	0 94	7 84	6 64	8 46

...s are given as mean values for seven subjects ± S.E. During the last min of exercise.

...the arm exercise. MBP remained unchanged and LVR increased on transition from ... exercise to combined exercise.

During arm exercise, on the other hand, the increase in (a-v)O diff in the exercising arms ... transition to combined exercise depended on the severity of the leg exercise (Fig. 4). Furthermore, a decrease in MBP was also related to the leg exercise.

The group mean lactate concentration values in all blood samples taken during the two ... min exercise periods are shown in Fig. 2. During combined exercise, where lactate concentrations increased steeply times for blood sampling presented in the figure are corrected ... the measured mean transit time in the legs and an estimated mean transit time in the arms ... 10 sec. For the first five min the shape of each curve was drawn according to previous ... (Klausen et al. 1974). In both arm and leg exercise there is an almost constant, ... (-a) difference in the exercising extremities. At the same time a negative (v-a) difference was seen in the resting extremities. On transition from leg exercise or arm exercise ... combined exercise there was steep increase in lactate concentration in all blood samples, ... no significant difference between the arterial and venous values.

Discussion

The main finding in the present study was that superimposing a sufficiently strenuous arm exercise on heavy leg exercise caused a reduction in blood flow as well as in oxygen uptake

TABLE II Respiratory and central circulatory data at rest and during three types of exercise

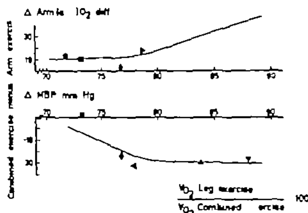
	$\dot{V}_{O_2}$, l × min⁻¹ STPD	V_E, l × min⁻¹ BTPS	V_{CO_2} × V_{O_2}	HR beats min⁻¹	$\dot{Q}$, l min⁻¹	Blood pressure, mmHg Systolic	Diastolic	mean	[illegible]
Rest									
Mean	0.29	9.7	0.95	76	10	133	77	96	[illegible]
S.E.	0.014	0.96	0.057	3.5	1.0	6.3	4.5	5.0	[illegible]
Arm exercise									
Mean	1.4	54	0.98	150	14	182	100	127	[illegible]
S.E.	0.14	5.8	0.026	6.3	1.5	7.0	1.8	3.3	[illegible]
Combined exercise									
Mean	3.4	120	0.97	185	23	172	85	114	[illegible]
S.E.	0.24	7.4	0.013	4.3	1.2	9.2	4.4	5.7	[illegible]
Difference	2.0	66	−0.01	35	9.0	−10	−15	−13	[illegible]
S.E.	0.10	3.2	0.018	4.5	0.55	5.9	2.7	3.0	[illegible]
t value	19.25	20.30	0.70	7.83	16.4	1.66	5.60	4.5	[illegible]
Leg exercise									
Mean	2.7	77	0.91	157	21	162	83	109	[illegible]
S.E.	0.13	5.3	0.015	9.3	1.0	8.1	3.8	5.0	[illegible]
Combined exercise									
Mean	3.4	124	0.99	189	[illegible]3	163	84	110	[illegible]
S.E.	0.23	9.6	0.011	4.0	1.7	9.0	5.5	6.2	[illegible]
Difference	0.7	47	0.08	32	[illegible]4	1	1	1	[illegible]
S.E.	0.12	6.8	0.013	9.0	0.68	5.0	4.1	4.0	[illegible]
t value	6.39	6.81	6.43	3.53	3.45	0.26	0.26	0.23	[illegible]

Data are given as mean values for seven subjects ± S.E.

as percentage of V_{O_2} max for the respective types of exercise, V_{O_2} during arm exerc[ise] was 44 (range 38–62) V_{O_2} during leg exercise 67 (range 58–78) and V_{O_2} during combi[ned] exercise 77 (range 71–83)

Table II and Table III give mean values for respiratory metabolic as well as for cen[tral] and peripheral circulatory variables measured at rest and during the exercise periods HR, $\dot{Q}$ and MBP at rest and during the three types of exercise were related to V_{O_2} as previo[usly] reported (Asmussen and Hemmingsen 1958 Bevegaard *et al* 1966, Stenberg *et al* 19[67] Clausen *et al* 1973) For all three variables the slopes of the linear relationships were the the same for leg exercise and combined exercise, while somewhat steeper slopes were se[en] during arm exercise Except for minor differences in HR and lactate concentration practically identical values were obtained during the *two periods of* combined exerc[ise] preceded by arm exercise or leg exercise, respectively

Leg blood flow was reduced by an average of 1 9 l × min when arm exercise wa[s] superimposed on leg exercise. The average arterio-venous oxygen difference ((a-v)O_2 d[iff]) in the exercising leg increased at the same time by 20 ml O × l The group mean va[lue] for the rates of oxygen uptake in the exercising legs was not significantly changed on the transition from leg exercise to combined exercise However as shown by the individual da[ta] presented in Fig. 3 LBF and oxygen uptake rate in the exercising legs became clear[ly] reduced when the arm V_{O_2} amounted to more than 40% of the total V_{O_2} during combined exercise. In contrast, the change in (a v)O diff in the legs was independent of the severi[ty]



...re 4 Effect of adding leg exercise to ongoing arm exercise. Individual changes in regional arterio-venous ... difference ((a-v)O_2 diff) and mean arterial blood pressure (MBP). Δ indicates differences ... lated in analogy to those of Fig 3. The abscissa is $\dot{V}_{O_2}$ for the leg load when performed separately ... pressed as percent of $\dot{V}_{O_2}$ during combined exercise. Levels fitted by eye.

...ussen 1968). Of specific relevance for our present findings is a study of Strandell and ...pherd (1967) who in man demonstrated a vasoconstriction in the active muscles of the ... arm performing mild to moderate dynamic exercise when subatmospheric pressure was ...ied to the lower part of the body. However, this sympathetic restraint on blood flow ... contracting muscles vanished during severe dynamic exercise. The findings in the ...sent study indicate that increased sympathetic activity obtained by superimposing ...rcise with extra muscle groups may counteract the metabolic vasodilation and mediate ...oconstriction even in muscles performing severe exercise. Thus, beyond a certain limit ...ork intensity and/or mass of active muscles, several muscle groups exercising at the ...me time will limit the oxygen supply to one another—*i.e.* each muscle group will have a ...er perfusion than if it performed its share of the total work load alone.

It is noteworthy that the restriction in muscle blood flow became manifest at a work ...ensity where $\dot{V}_{O_2}$ max was not yet attained. This affords strong support to the assump...on of a peripheral circulatory limitation for $\dot{V}_{O_2}$ max (Clausen 1976). Apparently the ...rvous control of the peripheral vessels prevents the occurrence of maximal vasodilation ...multaneously in major parts of the total skeletal muscle mass. The origin of this vaso...constriction is unknown. It might be suggested that the vasoconstriction in exercising skeletal ...muscles were brought about via receptors in the central circulatory system *e.g.* baroreceptors ...erving the maintenance of MBP. However, on transition from leg exercises to combined ...rcises the vasoconstriction sets in without a decrease in MBP or pulse pressure (Fig. 1) ...and at a work load where the absolute maximum for cardiac output is not attained.

Another possibility is that the vasoconstriction in exercising skeletal muscles is mediated by the same mechanism as the vasoconstriction in abdominal viscera which probably is elicited by receptors in the exercising muscles (Clausen *et al.* 1973, Rowell 1974, Clausen and Trap-Jensen 1974).

The reduction in driving pressure to the arms and the increased resistance to flow in the

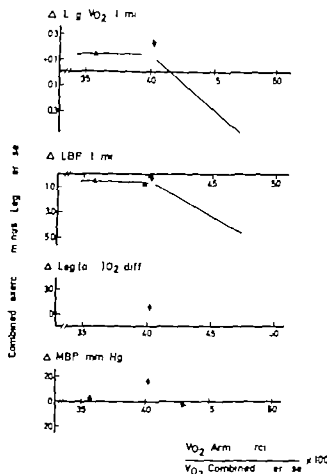


Fig. 3. Effect of adding arm exercise to ongoing leg exercise. Individual changes in leg oxygen uptake (leg $\dot{V}O_2$), leg blood flow (LBF), regional arterio-venous oxygen differences ((a-v)O_2 diff), and mean arterial blood pressure (MBP) ... indicates differences calculated by subtracting values obtained during separate leg exercise from values obtained during combined exercise. The abscissa is $\dot{V}O_2$ ... the arm load performed separately expressed as percent of $\dot{V}O_2$ during combined exercise. Lines fitted by eye.

in the exercising legs. Likewise the changes in (a v)O diff in the arms on transition from arm exercise to combined exercise conformed to a reduction in blood flow in the exercising arms. As judged from the changes in the regional lactate concentrations, blood flow changes affected metabolism in the arms to a greater extent than in the legs. There was a steep increase in lactate concentrations in arterial as well as in iliac and axillary venous blood on transition to combined exercise (Fig. 2). The (v-a)diff for lactate in the arms and in the legs may be taken to indicate that the increase in arterial lactate concentration during combined exercise was caused primarily by lactate release from the arm muscles. However it must be stressed that these arterial and venous lactate concentrations ... apply to unsteady state conditions.

The reduction in LBF on adding arm exercise to leg exercise occurred with no change in MBP. A decrease in flow with unchanged driving pressure implies an increase of the vascular resistance in the exercising legs (Table III Fig. 5). Part of this may be caused by reinforced sympathetic vasoconstriction in non-exercising tissues, but the decrease in oxygen uptake in the legs indicated that also the active muscles were affected.

Experiments performed on animals and in man indicate that the vascular resistance in exercising muscles results from a competition between a local metabolic dilator influence on resistance vessels and a general sympathetic vasoconstrictor activity (Mellander and

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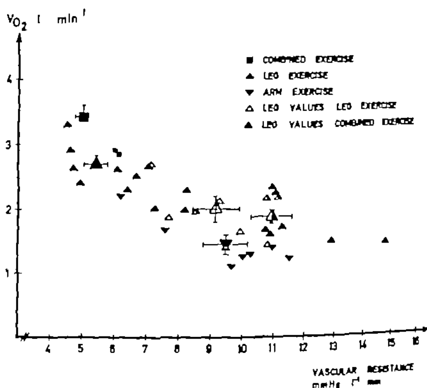


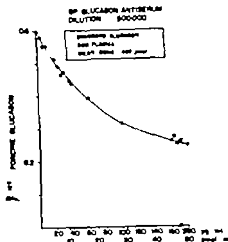
Fig. 5 Relationship between oxygen uptake (V_{O_2}) nd vascular resistance d ring arm, leg and com exercise, individual and group mean values ±S.E. Pulmonary oxygen uptake related to total va resistance (■ ▲ ▼) Leg oxygen uptake related to leg vascular resistance (△ ▵).

exercising legs during combined exercise provide two possible explanations for the that $\dot{V}_{O_2}$ during this type of exercise is not the algebraic sum of V_{O_2} during arm ex and V_{O_2} during leg exercise performed separately (Table 2, Fig. 5). At the same time may explain why relatively small augmentations are obtained in V_{O_2} max by adding with further muscle groups to that performed by large muscle groups.

This study was supported by Idrettens Forskningsråd. The authors are indebted to professor E. Asm August Krogh Institute, University of Copenhagen, Denmark for valuable comments and criticism

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1. Ratio of antibody-bound (B) to free (F) I[125] glucagon as a function of the concentration of unlabelled porcine glucagon and serial dilutions of plasma. The concentration in plasma samples was obtained from the average of all measured concentrations. The concentration in each dilution was calculated from the dilution factor and is plotted against the observed B/F ratio.

The food consisted of minced boiled beef liver suspended in water. In teasing experiments that were carried out for 5 min the dogs were allowed to see and smell the food but not to taste it. In some expts 0.2 mg/kg atropine sulphate (ACO, Sweden) was given 45 min prior to sham feeding. During basal periods and during stimulation blood samples were collected from a peripheral vein. The blood was centrifuged at [illegible] rpm for 10 min in a refrigerated centrifuge. After the plasma had been removed it was immediately frozen and kept at −20°C until assayed for glucagon concentration by radioimmunoassay. Plasma concentration of glucose was determined using Glox reagent (Kabi, Stockholm, Sweden). Antibodies to glucagon produced in guinea pigs using commercial glucagon (Lilly) for immunization were obtained from Carlo Patrono, Rome, Italy. The antiserum which was used in a final dilution of 1:500 000 permitted detection of 2 pg/ml of glucagon in buffer solutions. The gastrointestinal peptides secretin or VIP [illegible], which have structural similarities to glucagon, were added to solutions of antibodies and labelled glucagon in concentrations that were 10 000 times greater than the smallest amount of standard glucagon causing displacement of labelled glucagon. No displacement was observed upon addition of these hormones, thus indicating that the present antiserum does not cross-react with secretin or VIP.

Plasma samples were generally assayed at a dilution of 1:3. In recovery expts, porcine glucagon was added to plasma devoid of glucagon-like immunoreactivity. 90–112% of the added material was recovered in these expts. When a dog plasma sample containing a high concentration of glucagon-like immunoreactivity was tested at different dilutions the plasma dilution curve could be superimposed on the standard curve prepared in buffer solutions with porcine glucagon (Fig. 1).

To determine whether the used glucagon antiserum did cross-react with enteroglucagon present in plasma the pancreas was removed from a dog under Nembutal anesthesia (30 mg/kg). After the operation the dog obtained two subcutaneous injections of 12 U of NPH insulin and 6 U regular insulin (Vitrum, Stockholm, Sweden). Blood samples were collected on repeated occasions before the operation. After the operation further plasma samples were taken. Plasma concentration of glucagon and glucose (Glox reagent, Kabi, Stockholm, Sweden) were determined on the blood samples taken pre- and post-operatively. Glucose concentrations were in all samples within normal limits (80–90 mg/100 ml). Post-operative basal blood samples that were collected at the latest 15 h after the operation contained 8–11% of the concentration of glucagon-like immunoreactivity that was found in samples collected prior to operation.

Porcine glucagon (Novo) was used for labelling and as standard. Labelling was essentially carried out as described by Hunter and Greenwood (1962). At labelling, approximately 500 μCi (1 μl) of NaI[125] (Union Carbide) and 5 μl of glucagon (1 mg/ml) were added to a 0.25 M glycine buffer solution of pH 9.2 (20 μl). In sequence there were added 15 μl of Chloramine T (5 mg/ml), 20 μl of sodium metabisulfite (5 mg/ml) and 30 μl of outdated human plasma containing 500 units of Trasylol per ml (generously supplied by Bayer A.G.). To the final volume was added 0.5 ml of a solution of 0.02 M Veronal buffer (pH 8.6) containing 1 ml of guinea pig control serum, 1.25 ml of Human serum albumine (200 mg/ml) and 500 units of Trasylol

Acta physiol. scand. 1977 100, 298-302
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Effect of Teasing and Sham Feeding on Plasma Glucagon Concentration in Dogs

By

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Received 2 November 1976

Abstract

NILSSON G and K UVNÄS-WALLENSTEN *Effect of teasing and sham feeding on plasma glucagon concentration in dogs* Acta physiol scand 1977 *100* 298-302

Plasma glucagon concentration was determined by radioimmunoassay duri g fasting and following teasing and sham feeding. Teasing and sham feedi g f r 1-10 mi raised the plasma glucagon concentration which then within a short period of time returned to the basal level. Atropinization (0.2 mg/kg) almost abolished the glucagon response to 10 min of sham feeding. Glucose concentration was slightly elevated in response to 10 min of sham feeding. The results show that physiological stimulation that is induced by the smell, sight and taste of food releases glucagon in the dog The release is mediated by a cholinergic mechanism.

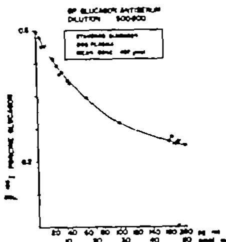
Key words Glucagon-teasing-sham feeding-radioimmunoassay

Recently it was demonstrated by electron microscopical technique that cholinergic nerve terminals are applied to the surface of the alpha cells (Renold 1971). Perfusion of the isolated pancreas in the dog with acetylcholine (Iversen 1973 Kaneto and Kosaka 1974) causes release of glucagon Also electrical stimulation of the vagal nerves has been shown to increase the plasma level of glucagon (Bloom, Edwards and Vaughan 1974 Kaneto, Miki and Kosaka 1974) Sham feeding in the dog causes release of gastrin (Nilsson et al 1972, Tepperman Walsh and Preshaw 1972, Nilsson 1975) and insulin (Nilsson and Uvnäs-Wallensten 1974) Such release is prevented by prior atropinization (Nilsson et al. 1972, Nilsson and Uvnäs-Wallensten 1974) indicating that cholinergic mechanisms are involved at stimulation by sham feeding. The present studies were undertaken to determine if stimulation induced by sham feeding releases glucagon in the dog. Plasma glucagon concentrations were determined by radioimmunoassay

Methods

Experimental technique

Dogs were provided with esophageal fistulae (Olbe 1959) t permit sham feeding. Before experiments were started the dogs had been fasted for 16-20 h. In sham feeding expts. that were performed for 1 or 10 min, the esophagus was obstructed below the esophageal cannula preventing the h the stomach.



1 Ratio of antibody-bound (B) to free (F) [125]I glucagon as a function of the concentration of unlabelled porcine glucagon and serial dilutions of plasma. The concentration in plasma sample was estimated from the average of all measured concentrations. The concentration in each dilution was calculated from the dilution factor and is plotted against the observed B/F ratio.

...'s food consisted of minced boiled beef liver suspended in water. In teasing experiments that were carried out for 5 min the dogs were allowed to see and smell the food but not to taste it. In some expts. 0.2 mg/kg atropine sulphate (ACO, Sweden) was given 45 min prior to sham feeding. During basal periods and during stimulation blood samples were collected from a peripheral vein. The blood was centrifuged at ...00 rpm for 10 min in a refrigerated centrifuge. After the plasma had been removed it was immediately frozen and kept at −20°C until assayed for glucagon concentration by radioimmunoassay. Plasma concentration of glucose was determined using Glox reagent (Kabi, Stockholm, Sweden). Antibodies to glucagon produced in guinea pigs using commercial glucagon (Lilly) for immunization were obtained from Carlo Patrono, Roma, Italy. The antiserum which was used in a final dilution of 1:500 000 permitted detection of 2 pg/ml of glucagon in buffer solutions. The gastrointestinal peptides secretin or VIP (...), which have structural similarities to glucagon, were added to solutions of antibodies and labelled glucagon in concentrations that were 10 000 times greater than the smallest amount of standard glucagon causing displacement of labelled glucagon. No displacement was observed upon addition of these hormones, thus indicating that the present antiserum does not cross-react with secretin or VIP.

Plasma samples were generally assayed at a dilution of 1:5. In recovery expts. porcine glucagon was added to plasma devoid of glucagon-like immunoreactivity. 90–112% of the added material was recovered in these expts. When a dog plasma sample containing a high concentration of glucagon-like immunoreactivity was tested at different dilutions the plasma dilution curve could be superimposed on the standard curve prepared in buffer solutions with porcine glucagon (Fig. 1).

To determine whether the used glucagon antiserum did cross-react with enteroglucagon present in plasma, the pancreas was removed from a dog under Nembutal anesthesia (30 mg/kg). After the operation the dog obtained two subcutaneous injections of 12 U of NPH insulin and 6 U regular insulin (Vitrum, Stockholm, Sweden). Blood samples were collected on repeated occasions before the operation. After the operation further plasma samples were taken. Plasma concentration of glucagon and glucose (Glox reagent, Kabi, Stockholm, Sweden) were determined on the blood samples taken pre- and post-operatively. Glucose concentrations were in all samples within normal limits (80–90 mg/100 ml). Post-operative basal blood samples that were collected at the latest 15 h after the operation contained 8–11% of the concentration of glucagon-like immunoreactivity that was found in samples collected prior to operation.

Porcine glucagon (Novo) was used for labelling and as standard. Labelling was essentially carried out as described by Hunter and Greenwood (1962). At labelling, approximately 500 μCi (1 μl) of NaI^{125} (Union Carbide) and 5 μl of glucagon (1 μg/ml) were added to 0.25 M glycine buffer solution of pH 9.2 (20 μl). In sequence then were added 15 μl of Chloramine T (5 mg/kg), 20 μl of sodium metabisulfite (5 mg/ml) and 30 μl of outdated human plasma containing 500 units of Trasylol per ml (generously supplied by Bayer A. G.). To the final volume was added 0.5 ml of a solution of 0.02 M Veronal buffer (pH 8.6) containing 1 ml of guinea pig antiserum, 1.25 ml of Human serum albumine (200 mg/ml) and 500 units of Trasylol

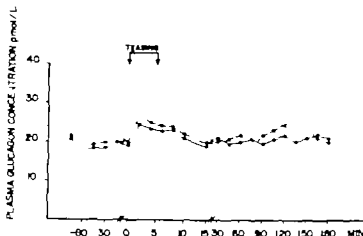


Fig. 2. Plasma glucagon concentration before and after 5 min teasing. Each curve represents one experiment.

per 1 ml. The mixture containing the labelled material was transferred to a 1.5 × 0.4 cm glass column packed with cellulose powder (Whatman).

Following repeated washings with destilled water the labelled glucagon was eluted with 1.5 ml of ethanol. To each fraction of 0.3 ml was added 0.5 ml of the Veronal buffer solution. This method for labelling and purification provided a labelled material of high specific activity and of excellent stability.

In assays tubes were incubated in cold room for 6 days. At separation 150 μl of outdated human plasma was added to tubes containing no plasma. The free antigen was then separated from the antibody bound by the addition of 200 μl of activated charcoal (50 mg per ml in 0.02 M Veronal buffer) and subsequent centrifugation. Both the supernatant tubes containing the antibody bound antigen and the charcoal tube containing the free antigen were counted in an automatic gamma scintillation counter.

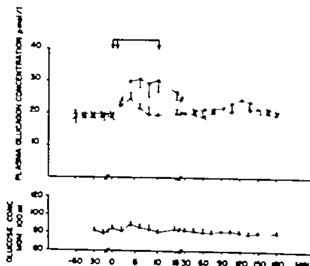
Results

The effect of teasing on plasma glucagon concentration was studied in 2 expts. This exposure to food slightly increased the plasma glucagon concentration (Fig. 2). Sham feeding for 1 min also caused a slight and short lasting elevation of the glucagon levels. The increase in plasma glucagon concentration was considerably more pronounced and sustained when sham feeding was prolonged. A late second elevation of the plasma glucagon level in response to sham feeding was seen in several expts. These results are illustrated in Fig. 3. Glucose levels were followed before and following 10 min of sham feeding. Sham feeding slightly increased the glucose level in plasma.

Atropinization did not significantly influence the basal plasma level of glucagon, whereas the glucagon response to 10 min of sham feeding was almost abolished (Fig. 4).

Discussion

The glucagon antiserum that was used in the present study does not cross-react with the gastrointestinal peptides secretin and VIP which have certain structural similarities with pancreatic glucagon. The antiserum cross-reacts with glucagon from intestinal extracts. However, only minor glucagon-like immunoreactivity could be detected in plasma samples from a dog that had undergone resection of the pancreas. Recently it has been demonstrated that removal of the pancreas rather rapidly causes an increase of the plasma con



3 Plasma glucagon concentrations before and after 1 (●—●) and 10 (○—○) min sham feeding. Each curve represents the mean of 9 expts in dogs. Glucose determinations performed on plasma samples expts here dogs were sham fed for 10 min. Vertical bars represent 1 S.D.

…tration of glucagon-like immunoreactivity (Matsuyama and Foà 1974, Vranic, Pek and …ranori 1974). Such an increase is reduced if animals are given insulin (Matsuyama and … al 1974). In one expt. the dog obtained injections of insulin that kept the plasma glucose …ncentrations at normal levels at occasions of blood sampling. This may explain why we …served low levels of glucagon-like immunoreactivity following pancreatectomy. Our …ntrol expt. performed therefore suggest that the antiserum used in the present study …entially reacts with plasma glucagon of pancreatic origin.

No reduction of basal plasma levels of glucagon following the injection of 0.2 mg/kg of …ropine was observed in the present study. In contrast, Bloom and Cow (1974) found a …duction of the plasma glucagon level in man after the administration of 15 μg of atropine …r kg b.wt. We have not either observed suppression of basal levels of gastrin (Nilsson et al …972) or insulin (Nilsson and Uvnäs-Wallensten 1974) in dogs following atropine. Unless …tremely high amounts of atropine are required to suppress the basal levels of gastrin,

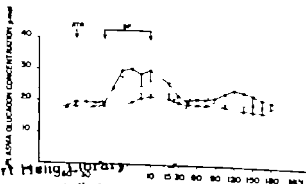


Fig. 4. Plasma glucagon concentrations before and after 10 min of sham feeding with (●—●) and without (○—○) atropinization. Each curve represents the mean of 9 expts. in 3 dogs. Vertical bars represent 1 S.D.

insulin and glucagon in dogs or unless our assays have not been able to detect changes o hormone concentrations induced by atropine the present results indicate that the bas release of these hormones is not influenced by atropine in the dog. Teasing and sham feeding however evoked release of the plasma concentration of glucagon. Since there is morphological evidence that the pancreatic islet tissue have a cholinergic innervation (Renold 1971 since the glucagon response to teasing and sham feeding is prompt and since atropine almost abolishes the glucagon response to sham feeding the results suggest that sham feeding initiates release of glucagon via a nervous, *i.e* cholinergic mechanism. Sham feeding al caused a slight increase in the plasma level of glucose. Sham feeding is a complex stimul that may activate several factors that influences the plasma concentration of glucose. The release of glucagon that was indicated in the present expts. may be one such factor

Following vagal activation by sham feeding glucagon, gastrin (Nilsson, Simon, Yalow and Berson 1972, Tepperman Walsh and Preshaw 1972) and insulin (Nilsson and Uvnäs-Wallensten 1974) are released into the blood in a similar pattern. The physiological signif icance of a sudden and considerable release of these hormones at the beginning of a me cannot at present be fully evaluated Possibly the released hormones initiate the process that are associated with digestion and metabolism of food components.

This study was supported by research grants from the Swedish Medical Research Council (project n 04X 3521)

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Acta physiol. scand. 1977. 100. 303–308

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Pressure-Independent Inhibition of Sympathetic Activity by Noradrenaline: Role of Baroreceptor C Fibres*

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Received 19 November 1976

Abstract

re, S. and H. AARS. *Pressure-independent inhibition of sympathetic activity by noradrenaline: role of baroreceptor C fibres.* Acta physiol. scand. 1977. *100.* 303–308.

effect of infusion of noradrenaline on activity in the renal sympathetic nerve as studied in bits anesthetized with chloralose and urethane. Noradrenaline (3–8 µg/kg min) initially increased mean erial pressure 20–40 mmHg and consequently reduced renal nerve activity. However, studies over e range of pressures—obtained by changing the blood volume, revealed that noradrenaline after minutes had induced pressure-independent reduction of sympathetic discharge. The effect disappeared th baroreceptor denervation. An unchanged relationship between arterial pressure and integrated tivity in the hole left aortic nerv (hich is largely measure of activity in A fibres) suggested that sympathetic depression was due to excitation of aortic nerve C fibres. This conclusion was supported studies of sympathetic responses to selective stimulation of aortic nerve A and C fibres at equal ssures before and during infusion of noradrenaline. Compared to the reflex activity from A fibres, fibre stimulation as invariably less effective in suppressing renal nerve activity during the infusion. e studies indicate that noradrenaline may effect negative feedback control of sympathetic discharge rough activation of baroreceptor C fibres.

ince first demonstrated by Palme in 1936, local application of adrenaline or noradrenaline the carotid sinus or aortic arch has been shown repeatedly to induce a marked reflex eduction in arterial blood pressure (Palme 1944, Heymans and Heuvel-Heymans 1950, Landgren, Neil and Zotterman 1952, Kalkoff, Pflechka and Rath 1966). The effect is presumably due to excitation of receptors with non-myelinated afferents, *i.e.* the C fibres. Activity the myelinated baroreceptor fibres (A fibres) is reduced. Landgren *et al.* (1952) and Peterson (1966) considered the catecholamine-activated fibres to be baroreceptor afferents, while Kalkoff *et al.* (1966) held them to represent a separate hypotensive reflex system. Recent data from our laboratory indicate that, at least in the aortic nerve in rabbits, C fibres are indeed engaged in the baroreceptor reflex—but only at pressures above normal

* This study was first presented at the XXVI International Congress of Physiological Sciences, New Delhi, Oct. 20–26, 1974

resting levels (Aars, Myhre and Haswell 1977). Electrical stimulation of these fibres induce severe inhibition of sympathetic vasomotor discharge and excitation of cardiac neurons, resulting in bradycardia and a fall in blood pressure (Douglas, Ritchie and Schaumann 1956, Kendrick *et al.* 1973, Kardon, Peterson and Bishop 1979).

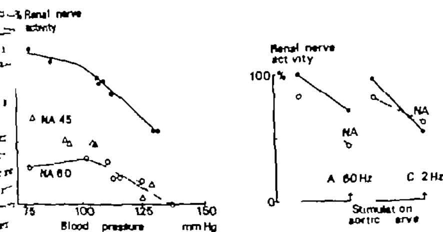
It would be of interest to know if adrenergic activation of the C fibres is restricted to local application of catecholamines, or whether it can be evoked by i.v. infusion of noradrenaline in doses producing only a moderate rise in blood pressure. If so, adrenergic excitation of baroreceptors with C fibres might pass from being considered an experimental phenomenon into having an important physiological function. To test this possibility, we have examined, in anesthetized rabbits, the relationship between blood pressure and sympathetic activity during infusion of increasing doses of noradrenaline. Noradrenaline was found to exert a pressure-independent reduction in sympathetic activity which, as judged by responses to selective electrical stimulation of aortic nerve A and C fibres, probably resulted from excitation of baroreceptors with C fibres.

Methods

The experiments were performed in 18 adult rabbits, anesthetized by a mixture of 1% chloralose (3 ml kg⁻¹) and 25% urethane (3 ml kg⁻¹) half i.v. half intraperitoneally. Free airways were secured by tracheotomy. Blood pressure was measured with a Statham transducer connected to a catheter in the right common carotid artery. A catheter in the right jugular vein was used for infusion of noradrenaline (NA). Sympathetic nerve activity was recorded from the intact left renal nerve, and baroreceptor activity from the intact left aortic nerve, by means of platinum electrodes and Tektronix 122 or Grass P 15 preamplifiers. Renal nerve activity was quantified by planimetry of average activity (Beckman 9852A), aortic nerve activity by rectification and integration of the signals (Aars and Leraand 1968). Output from the integrators was linearly related to frequency in the spectrum of activity found in the respective nerves. Mean nervous activity per second was expressed in per cent of activity at resting control blood pressure. Electrical stimulation of the left aortic nerve was made with square-wave pulses delivered from a Grass stimulator and an isolation unit. For differential activation of A and C fibres, the nerve was stimulated with pulses of 6 V 0.02 ms duration and 60–100 Hz (A fibres) or 6 V 1.5 ms and 2 Hz (A + C fibres) in periods of 10 s. The specificity of stimulation was controlled by studying the evoked action potentials cranial to the site of stimulation. The characteristics used to stimulate C fibres also stimulated A fibres but, in this low frequency range, reflex effects of the stimulation were solely due to activity evoked in the C fibres. All recordings were made on Sanborn or Beckman polygraphs.

Rabbits included in these series had control mean arterial pressure of 80–120 mmHg. The experiments began by recording renal and aortic nerve activities at various pressure levels, obtained by producing step-wise changes in blood volume or by inflating a balloon (Fogarty Arterial Embolectomy Cath 4 F) inserted through the left femoral artery into the descending part of the thoracic aorta. 30–60 s passed at each new pressure level before recordings were made. Arterial pressure was returned to resting control levels for recording of sympathetic responses to stimulation of the aortic nerve. The whole procedure was then repeated during infusion of NA, when blood pressure was again controlled by adjusting the blood volume. If more than one infusion was given to the same animal, the interval between infusions exceeded 30 min.

The effects of aortic nerve stimulation were evaluated, in each animal and for A and C fibre stimulation separately, by dividing the evoked reduction in sympathetic activity (average for whole stimulation period) during infusion of NA with the reduction obtained by the same stimulation and at the same blood pressure prior to administration of the drug. The ratio between C and A fibre effects, rather than the separate responses, was then used to assess the influence of NA on activity in aortic nerve C fibres. This procedure was necessary in order to minimize the effects of time-related variations in hemodynamics and reflex responses of the animals.



Inhibition of renal sympathetic nerve activity during constant rate infusion of noradrenaline (... doses in µg/kg min). Mean blood pressure controlled by changes in blood volume. Renal activity (100 ... at resting control pressure) integrated over 10 sec at each new pressure level. Observations control run and two infusion periods in one rabbit.

Reflex reductions of integrated renal sympathetic nerve activity in response to selective stimulation and A C (marked C, see Methods) fibres in left aortic nerve, before (full lines) and during infusion noradrenaline (NA, 8 µg kg min) in one rabbit. Mean arterial pressures: control 86 mmHg, with NA mmHg. Sympathetic activity: 100 ... activity before control stimulation A-60 Hz. Note reduced pre-stimulation activity during infusion of NA, and smaller effect of C fibre stimulation during than before infusion.

Results

...al nerve activity was largely unaltered by reductions in mean arterial blood pressure about 60 mmHg, but was sharply reduced when pressure was elevated above resting ...trol levels. Infusion of NA initially increased the blood pressure 20–40 mmHg and de... renal nerve activity. With blood pressure adjusted to pre-infusion levels, NA 2–4 min ...er start of infusion led to a reduction in sympathetic activity. This sympathetic inhibition ...creased with increasing doses of NA, and was found to exist over the full range of ...ssure and be maintained throughout the infusion period (Fig. 1). Individual sensitivity ...ried, but with doses of NA ranging from 3 to 8 µg/kg min, renal nerve activity in the 18 ...imals was reduced 17–78 ... t resting control mean blood pressures. Exact thresholds ...re not determined. After sectioning of the aortic nerves and occlusion of both common ...rotid arteries, renal nerve activity in 2 rabbits rose to 170 and 240% of control and ...came insensitive to infusion of NA.

As illustrated in Fig. 2, selective stimulation of aortic nerve A fibres induced about the ...me inhibition of renal nerve activity before, as during infusion of NA, but C fibre stimu...lation was less effective in suppressing renal nerve activity during administration of the ...ug. This reduced effect of C fibre stimulation was found in all but one animal, whereas ...e A fibre effect increased in three and decreased in 2 rabbits. However in the animal ...owing increased response to activity in C fibres, the response to A fibre stimulation was ...creased even more by NA. Animals with decreased A fibre effects displayed even greater

reductions in C fibre responses. Consequently the ratio between effects of NA on C and A fibre responses was invariably smaller than 1 0, on average 0 47 ± (S.D) 0.21 (64 ... in 5 animals)

The relationship between blood pressure and aortic nerve activity was examined in ... animals. Baroreceptor activity was unaffected by NA in 4 and reduced in 5 of them.

Discussion

Intravenous infusion of noradrenaline was found to induce a pressure-independent dec... in sympathetic nerve activity This finding conforms with the changes observed in ca... sympathetic fibres in cats following injection of adrenaline (Marguth, Raule and Sch... 1951) but contrasts with the results of other investigations, also in cats (Iggo and V... 1962, Weidinger Fedina and Kehrel 1963) The conflicting results are presumably du... differences in drug administration and experimental procedures. Iggo and Vogt (19... recorded activity in the cervical sympathetic nerve, which in our experience is a p... measure of the baroreflex. Weidinger *et al* (1963) injected adrenaline in smaller do... than did Marguth *et al* (1951) The time factor might also be important. If as will s... sequently be discussed the pressure-independent action of NA was mediated by excita... of baroreceptors with C fibres, it should be remembered that receptor effects of local ap... plication of catecholamines to the carotid sinus are known to develop over a few second... (Landgren *et al* 1952, Witzleb 1953 Kalkoff *et al* 1966) The same applies for the maxi... mum aortic constrictor response to i v administration of NA (Aars 1971 a, 1972 b) ... the present investigation, the sympathetic depression became evident 2-4 min after st... of the NA infusion This dependency on time for development of the effect of NA on bar... receptors probably explains why the effect was sometimes not elicited by brief injection... of catecholamines (Iggo and Vogt 1962, Weidinger *et al* 1963) In contrast, stimulati... of sympathetic fibres to the carotid sinus baroreceptors may induce an almost immedia... rise in receptor activity and fall in blood pressure (Palme 1944 Kezdi 1954, Koizumi ... Sato 1969 Sampson and Mills 1970 Wurster and Trobiani 1973)

The depressive effect of NA on sympathetic activity disappeared after exclusion of inp... from baroreceptor nerves This indicates that NA had mainly affected the baroreceptor... and that the sympathetic inhibition was not due to actions of NA on central pathways o... the reflex or on sympathetic ganglia.

In our expts. and in agreement with earlier results (Aars 1971 a), NA did not increa... the whole-nerve baroreceptor activity at any pressures. Since recordings of whole-ner... activity are dominated by activity in A fibres, activity in C fibres being masked, this mea... that the NA induced sympathetic inhibition was probably not caused by a rise of acti... in A fibres. In support of this conclusion present doses of NA have been shown to effect... slight reduction in the number of spikes per pulse beat in single A fibres of the rabbit... aortic nerve Heart rate was unaffected (Aars 1971 b) The sympathetic inhibition accordin... must have been due to increased discharge in afferent C fibres. This conclusion is in a... ment with previous demonstrations in few-fibre preparations ... reased C fibre...

...pike—activity following application of catecholamines to the carotid sinus (Land... *al.* 1952, Witzleb 1953 Kalkoff *et al.* 1966, Peterson 1966), and could be confirmed ...ent results of separate stimulation of A and C fibres in the aortic nerve. The effect ... passage" nerve stimulation depends upon many factors and is difficult to interpret. ...fferent nerve, the level of background activity is important the higher the back... ...l activity the greater the chance of impulse collision. Hence, the net increase in ...t traffic during electrical stimulation of the aortic nerve presumably will be smaller in ...ses with high than low background activity Centrally the outcome of stimulation ...intact aortic nerve is complicated by the addition of non-rhythmical afferent activity ... normal, pulse-synchronous input from several baroreceptor nerves. Most likely ...er, the net reflex effect of added afferent activity will be less when background ...y is high than when it is low This in particular holds for the C fibres, which pre...ably are silent at present control pressures, and whose sympathetic response curve ... very steep part at low stimulation frequencies. Aars *et al.* (1977) found that, in ...s, the sympathetic inhibition by C fibre stimulation of the aortic nerve reached 90% ...ximum already at 4 Hz stimulation maximum was found at 10–20 Hz. We therefore ... the sympathetic inhibition by aortic nerve stimulation to assess the effect of NA on ...receptor activity Having established that A fibre activity was largely unchanged, ...increased by present doses of NA, we introduced the response to stimulation of A ...as a reference to correct for other non-specific influences on the reflex during the ...iment. Since the stimulations were performed at the same blood pressure before, as ...ng infusion of NA, our demonstration of invariably smaller responses to C than A ...stimulation, *i.e.* a reduced C/A ratio implies that NA had induced a pressure ...pendent excitation of receptors with C fibres in the aortic nerve.

...conclusion, we have found that i. infusion of NA may cause a pressure-independent ...ction in sympathetic discharge by increasing the activity in baroreceptor C fibres. In ...tions with greatly elevated sympathoadrenal activity—like hypoxic and hypotensive ...s or fright reaction, this adrenergic effect on baroreceptors most likely would exert a ...sure-independent negative feedback control of sympathetic neurons.

...work ... supported by Anders Jahre's Fund for the Promotion of Science.

...quests for reprints should be addressed to Harald Aars, Box 1052, University of Oslo, Blindern, ... 3.

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Acta physiol. scand. 1977 100, 309–314
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the Cation Exchanger Properties of Rat Mast Cell Granules and their Storage of Histamine

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Received 2 December 1976

Abstract

is, B and C. H. Åborg. *On the cation exchanger properties of rat mast cell granules and their storage of histamine* Acta physiol. scand. 1977 *100* 309–314

cell granules free of surrounding membrane were isolated from water-lysed rat peritoneal and ic mast cells by differential centrifugation. The granules were depleted of their histamine by ation in 10 mM sodium phosphate buffer and the sodium-charged granules then converted into the ogen form" by repeated washing in slightly acid deionized water The cation exchanger properties mast cell granules were investigated by testing the applicability of the Rothmund-Kornfeld equation tion exchangers to the binding of Na and H ions to granule sites. The results lend further support view that the mast cell granule acts as cation exchanger with the exchanger function localized to n carboxyls in the protein-heparin complex of the granule matrix.

Abbreviations

histamine		NA	= noradrenaline
5-hydroxytryptamine		A	adrenaline
tyramine		PHC	= protamine-heparin complex
phenylethylamine		TrpA	= tryptamine

granule matrix of rat peritoneal mast cell consists mainly of two components, basic peptide and the acid polysaccharide heparin (Lagunoff *et al.* 1964 Bergqvist *et al.*). These two components form a complex, which is rather insoluble in water but h dissolves in 1 2 M NaCl. Titrations of intact and of dissolved granules indicate an c linkage between the acid groups of heparin and the basic groups of the polypeptide, protein carboxyls as the main free anionic sites of the complex (Uvnäs *et al.* 1970). ast cell granules without surrounding membrane can be isolated from mast cells lysed deionized water (Uvnäs *et al.* 1970). Such granules have the ability to take up and retain inorganic cations (Na Ca++ Zn++) and biogenic amines (PhEA TA DA NA etc.). Inorganic and organic cations compete for the same granule sites (Bergendorff and ls 1972). The narrow pH range (between 4-7) over which cation binding takes place, selectivity of this binding as well as the good agreement between the cation binding

$$\frac{C_{B_r}}{C_{A_r}} \times \left(\frac{C_{A_w}}{C_{B_w}}\right)^{\beta} = K$$

C_{B_r} and C_{A_r} = conc in resin phase
C_{A_w} and C_{B_w} = conc. in the external solution
β and K = empirical parameters

Fig. 1 The Rothm ndh-Kornfeld equation.

capacity and the number of titrable acid groups in the granules between pH 4-7 led to the conclusion that the heparin-protein complex in the granule matrix acts as a weak cation exchanger the COO groups of the protein acting as the ionic binding sites (Fig 4

The Rothmund Kornfeld equation (Fig. 1) is an empirical modification of the L Mass Action valid for cation exchangers (Samuelsson 1952).

According to the above equation when two cations compete for the ionic sites exchanger a plot of the log of the ratio between the two cations in the exchanger vs log of the corresponding cation ratio in the solution will yield a straight line. In the experiments we tested the applicability of the Rothmund Kornfeld exchanger equ the cation binding behaviour of the mast cell granules.

Methods

Mast cells were isolated from the abdominal and thoracic cavities of male Sprague-Dawley rats in deionized water The membrane-free granules obtained by differential centrifugation of the material were depleted of their amines nd sodium. To determine their cation binding prop granules were then suspended t the desired pHs in cation-containing media with appropriate of radioactive isotopes.

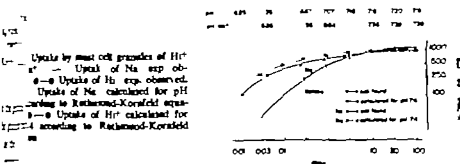
Details of the procedures used for the quantitative determination of the cation uptake by the have been described previously for Na and HI by Uvnäs *et al* (1970), for TA and 5-HT by B and Uvnäs (1972), for Zn^{+} by Uvnäs *et al.* (1975) and for PhEA TA, DA, NA A^{+} etc. by B a d Uvnäs (1973).

Materials

Histamine-(ring-2 ¹⁴C) d hydrochloride, sp.act. 51 mCi/mmol tyramine-1-¹⁴C hydrochlor 44 mCi/mmol dopamine-¹⁴C hydrochloride, sp.act 55 mCi/mmol DL-noradrenaline-(ca DL-bitartrate, sp.act. 28 mCi/mmol DL-adrenaline-(carbinol ¹⁴C) DL-bitartrate, sp act. 27 acetylcholine (methyl- ¹⁴C) chloride, sp-act. 54 mCi/mmol ²²NaCl, sp.act. 1 mCi/mg; ¹⁴C 4 mCi/mg. The Radiochemical Centre, Amersham, England 5-Hydroxytryptamine-2 ¹⁴C sp.act. 17 mCi/mmol tryptamine-2-¹⁴C bisuccinate sp.act. 8.9 mCi/mmol β-phenyl-ethyla hydrochloride, sp.act. 3 6 mCi/mmol, New England Nuclear Boston, Mass., USA. Pharmacia, Uppsala, Sweden. Human serum albumin (free from preservatives), AB Kabi, Sweden. All other substances were obtained from usual commercial sources.

Results and comments

Mast cell granules were depleted of their histamine by suspending them in 10 mM buffer at pH 7 4 The sodium-charged granules were then depleted of their so converted into the "hydrogen form" by repeated washing in deionized water (Uv 1970) This round-about procedure involving sodium exchange at pH 7 4 had to since in the slightly acid deionized water (pH 6) histamine became partly div



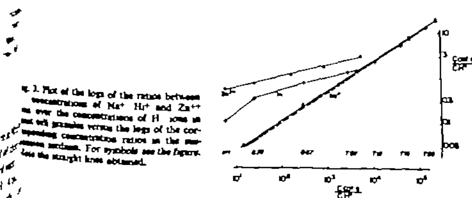
Uptake by mast cell granules of Hi⁺ ... Uptake of Na exp. observed ... Uptake of Hi exp. observed. Uptake of Na calculated for pH ... according to Rothmund-Kornfeld equation ... Uptake of Hi⁺ calculated for ... according to Rothmund-Kornfeld ...

to the fact that its affinity for granule binding sites is greater than that of hydrogen it could not be completely removed merely by washing in this water

... uptake of sodium and histamine was studied on mast cell granules prepared as ...ribed above. The two cations were taken up and retained in a dose-dependent manner in a concentration range of 0.001–30 meq/L thus confirming our previous results ...als et al. 1970). The uptakes for the two cations reached the same maximal level—for ...t batches of granules (1 000 neq/mg dry granule weight (Fig. 2).

... uptake studies on histamine and sodium depleted granules, the two cations competing ... the binding sites will be H ions and Na or Hi⁺ ions respectively. Since, as shown by ...tion (Uvnäs *et al.* 1970), the maximal uptake of H ions by the granules is the same as ... for Na and Hi⁺ ions (1 000 neq/mg), determinations of pH in the suspension ...ium and of the uptake by the granules of Na and Hi⁺ ions allow the applicability of ... exchanger equation to be tested on our experimental data.

As seen in Fig. 3, plotting of the log of the cation ratio in the granules versus the log ... the cation ratio in the suspension medium yields a straight line for Na over the whole ... range. For Hi a straight line, parallel with and close to the Na line was obtained ...ly for the uptake values at pHs above 7. The deviation of the Hi⁺ line below pH 7 is ...plained by the fact that below this pH histamine changes from being monovalent and ...comes increasingly divalent with falling pH, whereby its affinity for the granule binding ...es increases.



...g. 3. Plot of the logs of the ratios between ... concentrations of Na⁺, Hi⁺ and Zn⁺⁺ ...ons over the concentrations of H ions in ...ast cell granules versus the logs of the corresponding concentration ratios in the suspension medium. For symbols see the figure. ...ote the straight lines obtained.

Due to the high pH-sensitivity the binding of Na^+ and Hi^+ ions is practically abolished by lowering the pH of the suspension medium from 7 to 4. We therefore tried to ensure a constant pH of 7.4 in the suspension medium throughout the concentration range in the uptake studies. For the Na^+ uptake this was accomplished by using sodium phosphate buffer and in the corresponding Hi^+ studies by using an appropriate mixture of Hi base and Hi hydrochloride. However, as is evident from the pH values listed in Fig. 1 the buffering capacities were insufficient to keep the pH constant. With increasing dilution of the stock solutions the pH fell towards 6. The pH-dependent reduction of the uptake of the two cations is evident from Fig. 2, in which the experimentally found uptake curves for Na^+ and Hi^+ have been recalculated for pH 7.4 according to the Rothmund-Kornfeld equation. Such recalculated curves run practically identical courses, indicating the same affinities of Na^+ and Hi^+ ions for the granule binding sites, as shown in Fig. 3. The divalent cations Ca^{++} and Zn^{++} behave in principle like monovalent ions but have considerably higher affinities for the granule binding sites, as shown for Zn^{++} in Fig. 3.

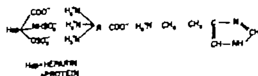
Discussion

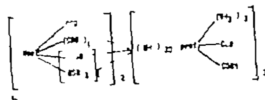
The main aim of the present investigation was to obtain further experimental evidence to illustrate the cation exchanger properties of the mast cell granules. The applicability of the Rothmund-Kornfeld equation for cation exchangers to the uptakes of Na and Hi corroborates the view that the granules have such properties.

We have previously suggested that histamine release from mast cells occurs as a consequence of the exocytotic process when membrane free granules become exposed to the cation-containing extracellular fluid. Since Na^+ and Hi ions have the same affinity for the granule binding sites (Fig. 2 and 3) and the sodium concentration in the extracellular fluid (150 meq/L) is manifold higher than that needed for saturation of these sites (3–10 meq/l, see Fig. 2) it follows that granules exposed to the extracellular fluid at neutral pH will be practically instantaneously depleted of their histamine by cation exchange. The main exchange will probably be with Na ions, but since according to Bergendorff and Uvnäs (1973) the divalent Ca^{++} ion has a considerably higher affinity for the granule binding sites (about 13 times higher than the Na^+ ion) also Ca^{++} ions may play a part in the histamine release, even though they are present in low concentration in the extracellular fluid. If we assume a 50% ionization of the extracellular calcium at a prevailing concentration of 5 meq/l, the Ca^{++} ions present should suffice to saturate the granule binding sites if they were the only cations present. However, in the extracellular fluid coming into contact with the granules the Ca ions have to compete with Na which are present there at more than 50 times the concentration.

Comments on the localization of the histamine binding sites

As discussed in several previous papers (see Uvnäs *et al.* 1970, Uvnäs 1974) we believe the cation exchanger function of the mast cell granule to depend on free carboxyl groups in the heparin-protein complex that forms the matrix of the granule. The endogenous histamine is thought to be stored in ionic linkage to these groups (Fig. 4).





4 a and b. Assumed gross-structure of protein-heparin complex of mast cell granules (from Uvnäs et al. 1970), b) an in vitro formed protamine-heparin complex, PHC at 7 (from Uvnäs and Åborg 1976).

b

From metachromatic titrations of mast cell granules *in vitro* with Acridine Orange and thylene Blue Lagunoff (1974) recently concluded that the capacity of the granules to bind se dyes was too large to be explained by ionic binding to protein carboxyls only. He ued that part of the dye binding must have occurred at negative sites of heparin, presumably at both carboxyl and sulphate groups. By analogy these acidic groups of heparin were sidered by Lagunoff as probable binding sites also for endogenous histamine. As will be cussed below e doubt the validity of this analogy and maintain our proposal that the tein carboxyls are the most likely histamine storage sites.

According to presently generally accepted view the mast cell histamine is thought to be stored in the granules in ionic (divalent) linkage to the strongly acid sulphate groups of the heparin. This assumption is based on the observation that in vitro heparin and histamine may form rather stable salt complex. The complex formation is optimal at pH 2–3 and at this pH the heparin content of the mast cell granule would suffice for the binding of all the endogenous histamine. However at pH around neutrality which is more likely to exist in vivo, the binding capacity of heparin is much less and the granule heparin would suffice to bind only ~10 per cent of the granule histamine.

As discussed in previous article (Uvnäs 1974) it is doubtful whether any acid groups of the granule heparin are available for cation binding. In the granule matrix the heparin occurs together with basic low molecular polypeptide. These two strongly charged components form stable salt complex (Lagunoff et al. 1964, Bergqvist et al. 1971). 1–2 M KCl solution is needed to break the salt linkages and dissolve the granules. From titrations on undissolved and dissolved granules, and of corresponding amounts of heparin we concluded that protein carboxyls were the only acid groups available for cation binding in intact granules. In other words, all the acid groups of the heparin carboxyl as well as sulphate groups should be occupied by basic groups of the protein (Fig. 4 a).

Indirect support for our view was obtained from studies on PHC, an in vitro formed ionic complex between heparin and the low molecular weight basic polypeptide protamine. The PHC behaved in all respects like cation exchanger with the terminal carboxyls as the sole cation binding sites. For Na⁺ and Hi⁺ ions the uptake curves approached but never surpassed the maximal uptake expected from the calculated numbers of free protamine carboxyls. Removal of these carboxyls did not prevent the complex formation but completely abolished its cation binding ability at pH 7.4.

In contrast to Na⁺ and Hi⁺ aromatic amines such as PhEA, NA and A showed a two-phase uptake. The first phase which occurred within the lower concentration range of up to 10–30 mmoles/l corresponded to the uptake of Na⁺ and Hi⁺ described above. However with increasing concentrations of the aromatic amines a second uptake phase began, probably due to the intrusion of these amines into the heparin-protamine ionic binding, primarily the carboxyl-amino linkages (Fig. 4 b). The mast cell granules also show two-phase uptake for certain amines, e.g. 5-HT and TrpA (to be published) and for heterocyclic amines

like chlorpromazine and toluidine blue the uptake considerably exceeds the number of protein carboxyls available for binding (Popova *et al.* 1970).

Metachromasia is a characteristic phenomenon which is seen on staining mast cell granules with certain basic dyes. The metachromatic spectral shift is assumed to be due to the binding of the dye to a polymer anion—in the mast cell granule this polyamine is heparin (for reference see Lagunoff 1974). The presence of metachromasia of the mast cell granules is therefore difficult to explain unless acid groups of heparin are available for dye binding. Accordingly in his metachromatic titrations Lagunoff revealed more [illegible] sites—probably mainly carboxyls—than the number of protein carboxyls expected from the calculations of Bergqvist *et al.* (1974).

We agree with Lagunoff when he argues that these metachromatic binding sites are acidic heparin groups. Both Acridine Orange and Methylene Blue are heterocyclic amines and in our experience such amines, like certain other amines (indolamines in PHC, aromatic amines in mast cell granules) have the ability in appropriate concentrations to break some of the ionic linkages between heparin and basic polypeptides and to bind to heparin carboxyl and possibly also sulphate groups thereby made available for binding.

Na^+ and H^+ ions do not seem to have the ability to unmask binding sites in the heparin part of the complex. The uptakes of these cations by the granules do not exceed the calculated number of available protein carboxyls, even in concentrations up to 100 mmoles/l, which are manifold higher than those required for saturation of these groups (3 mmoles/l). Therefore we still prefer to regard the mast cell granule as a cation exchanger with its exchanger function depending on protein carboxyls in the protein-heparin complex of the granule matrix. In our view endogenous histamine is stored in ionic linkage to these carboxyls, while the metachromasia phenomenon can be explained by the property of certain basic dyes like Acridine Orange, Methylene Blue and Toluidine Blue—which all are heterocyclic amines—to penetrate into the protein-heparin complex and bind to some of the acidic heparin groups.

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Acta physiol. scand. 1977. 100. 315–324

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Muscle Adaptation to Extreme Endurance Training in Man

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Received 30 December 1976

Abstract

Jansson, E. and L. Kaijser. *Muscle adaptation to extreme endurance training in man.* Acta physiol. scand. 1977 *100* 315–324

...evaluate the effect of extreme endurance training on muscle fibre composition and activities of oxidative enzymes in different fibre types biopsies were taken from vastus lateralis, gastrocnemius and deltoideus of ... orienteers. Comparisons were made between the (trained) leg muscles and the (relatively untrained) arm muscles, and with leg muscles of 16–18 years old boys. The orienteers had the same percentage type I ... in vastus lateralis and gastrocnemius as in deltoideus, but higher percentage type I fibres in vastus lateralis compared with the controls. The similarity between trained and untrained muscle in the orienteers suggests that training had not caused the high percentage type I fibres which rather might be the result of selection of individuals with the best prerequisites for high oxidative capacity. However the distribution of type II subgroups in the leg muscles of the orienteers differed from both their own deltoideus and leg muscles of the controls, the relationship IIA/IIB being altered in favour of the more oxidative IIA. The leg muscles of the orienteers also showed an increased occurrence of the normally rare IIC fibre. These latter findings point at the possibility of a training induced alteration in the subgroup pattern. Unlike in the controls there was no significant difference in succinate dehydrogenase activity, measured in single fibres, between type I and II fibres in gastrocnemius of the orienteers. Thus, type II fibres have the ability metabolically to adapt to high oxidative demands. This might to some extent be mediated by a conversion from IIB to IIA form.

Key words: Athletes, endurance, muscle fibre types, muscle enzymes, physical training

In recent years a number of studies, in both animals and man, have revealed that physical training of an endurance type results in increased activities of enzymes in Krebs cycle (Gollnick *et al.* 1973, Holloszy 1975, Saltin *et al.* 1976). In cross-country skiers increased activity of an enzyme involved in fat oxidation has also been described which indicates an increased capacity of fat oxidation (Bass *et al.* 1976). Thus, the skeletal muscle has the ability to adapt its metabolic capability to the increased demands of oxidation. Human skeletal muscle is, based on histochemical stainability for myofibrillar ATPase, made up of two main types of fibres. Type I fibres generally have high oxidative potential and type II high glycolytic capacity. Adaptation to high oxidative demands would be facilitated if both type II and type I fibres are able to markedly increase their oxidative capacity or if type II fibres could convert to type I fibres.

That fibre type conversion is theoretically possible has been shown in animal experiments where muscles have been electrically stimulated (Stréter *et al* 1973) or crossinnervated (Bárány and Close 1971) It has, however never been possible to show alterations in the percentage distribution of type I and type II fibres as a result of physical training in man (Gollnick *et al* 1973 Saltin *et al* 1976) although changes in cross-sectional area of the muscle fibres have been demonstrated (Saltin *et al* 1976) Still, successful athletes in endurance events are regularly found to have a high percentage type I fibres, though this may not necessarily be due to their training, but merely the result of selection of those individuals with the best prerequisites for high oxidative capacity (Gollnick *et al* 1972, Karlsson *et al* 1975 Hedberg and Jansson 1976)

The present study deals with three major questions concerning the influence of extreme endurance training on the characteristics of the human skeletal muscle.

(1) Do endurance trained athletes show an altered quantitative relationship between type I and type II fibres and are deviations to be found in the relative proportions of the subgroups IIA, IIB and IIC? This has been investigated by comparing a group of endurance trained men and a control group

(2) Are there any indications that an altered fibre composition is induced by training. This was studied by comparing trained and untrained muscles in the endurance trained subjects.

(3) Does endurance training elevate the oxidative capacity by increasing the activity of oxidative enzymes also in the generally less oxidative type II fibre?

In addition, we have studied if different modes of endurance training, so called "distance" and "interval" training differently affect the levels of muscle enzyme activity

Subjects and Methods

Eight Swedish elite orienteers took part the tudy Mean values of ge, height and weight were 4 (19–?) years, 180 (174–185) cm and 69 (64–74) kg, respectively Their maximal oxygen uptake determined on the bicycle ergometer averaged 5 0 (4 5–5 7) 1 min The subjects were divided i to two different groups according to the type f physical training. One group (4 subjects) used t run on the average 1?0 km and at moderate pace, a mode of training usually referred to as extreme distance training (group D). The other group utilized both "interval" nd, to moderat degree, distance training (group I). Group D and I did not differ significantly with regard t age, height weight and maximal oxygen uptake.

Muscle samples were taken from the quadriceps femoris (vastus lateralis, n = 8), the gastrocnemius (n = 8) and the deltoideus muscle (n = 4) with the needle biopsy technique (Bergström 1962). The samples from vastus lateralis and gastrocnemius were divided into three portions. Two of these were frozen immediately in liquid nitrogen and stored in − 80°C until analyzed quantitatively for enzymes. These enzyme analysis procedures were of two kinds. (1) One frozen muscle piece was homogenized (dilution 1 100) in 0 3 M phosphat buffer (pH 7 7) with 0.05 M BSA and was analyzed for succinate dehydrogenase (SDH, EC = 1.3.99 1), 3-hydroxyacyl-CoA-dehydrogenase (HAD EC = 1 1 1.35) and phosphofructokinase (PFK, EC = 2.7 1 11). *SDH analysis* 10 μl of the muscle h mogenat was incubated in 75 μl of the homogenization medium described above, containing 0 5 mM phenazine methosulphate and 0 15 M Na-succinate, for 30 min at 37°C in a water shake bath in darkness. The reaction was stopped by 75 μl 1 M NaOH and 75 μl brombenzol, mixed and centrifuged for 5 min. A quantitative determination of the produced malate and fumarate was then performed on the superantant (Essén *et al* 1975). *HAD-analysis*: 25 μl of the homogenate was added to 1 ml of the reagent solution earlier described (Essén *et al.* 1975) and the activity was expressed as μmol 3-hydroxyacetoacetyl-CoA produced per g muscle (wet weight) and min *PFK analysis*: 2 μl f the homogenate was added to 1 ml of the reagent solut n earlier described (Essén *et al.* 1975) (2) The other frozen piece was freeze-dried and SDH analyses were performed on outdissected single fragments of characterized muscle fibres (Essén *et al.* 1975).

Table I. *Percentage distribution of fibre types in vastus lateralis, gastrocnemius and deltoideus of the* orienteers and of vastus lateralis of the control subjects (Hedberg and Jansson 1976).

	n	Type I	Type IIA	Type IIB	Type IIC	No. of fibres counted/subject
Group D + I						
vastus lateralis	8	68.1 ± 8.4	24.4 ± 10.4	3.3 ± 4.0	4.2 ± 4.7	275 ± 137
gastrocnemius	7	67.1 ± 8.7	28.9 ± 8.9	1.9 ± 4.3	2.1 ± 3.1	242 ± 93
deltoideus	4	68.3 ± 11.8	14.3 ± 6.5	17.4 ± 6.6	0.0 ± 0.0	490 ± 169
Control group						
vastus lateralis	49	*53.9 ± 12.2*	*32.2 ± 9.1*	*13.0 ± 7.6*	*0.9 ± 2.1*	*376 ± 125*

Values are means ± SD. } denotes that p < 0.01

The third portion was mounted in cryoform embedding medium, frozen in isopentane at −160°C, sectioned and stained for myofibrillar ATPase after preincubations at different pH (Padykula and ... 1955, Brooke and Kaiser 1970), NADH-dehydrogenase (Novikoff *et al.* 1961) and mitochondrial glycerophosphate dehydrogenase (Wattenberg and Leong 1960). Fibre typing was based on the myofibrillar ATPase stain and fibres were classified as type I, IIA, IIB and IIC (Brooke and Kaiser 1970). Samples from the deltoideus muscle were analysed histochemically only.

The cross-sectional *areas of 20–25 fibres of each type (I and II) were estimated from the NADH-dehydro*genase stain by a grid method (Edström and Torlegård 1968/69).

A group of 16–18 years old boys of varying physical fitness was used as control group. Muscle samples were taken from vastus lateralis and analysed for the enzymes SDH (n = 53), HAD (n = 51) and PFK (n = 49) on muscle homogenate, fibre types (n = 49) and fibre areas (n = 62) (Hedberg and Jansson 1976).

Results

The orienteers had a high percentage type I fibres in both vastus lateralis (68.1 ± 8.4) and gastrocnemius *(67.1 ± 8.7)*, but also in the relatively untrained arm muscle deltoideus (68.3 ± 11.8) (Table I). The figures can be compared with the mean value 53.9 ± 12.2 % of type I fibres in vastus lateralis, in the control group. No significant difference was found between group D and I with regard to the percentage type I fibres.

The percentage distribution of subgroups of the type II fibres in vastus lateralis and gastrocnemius among the orienteers also differed from the controls. Thus the quantitative relationship between IIA and IIB was altered in favour of type IIA, a fibre type that normally has higher oxidative capacity than IIB (Table I and Fig. 1). Some of the orienteers, particularly in group D had no IIB fibres at all or only a few per cent. An increased occurrence of the rather highly oxidative IIC was seen (Table I, Fig. 2 and Fig. 3). This latter fibre is rarely found in muscles of the control subjects. M. deltoideus of the orienteers had a high percentage II B and no IIC fibres like the leg *muscles of the controls.*

The mean areas of type I and type II fibres, respectively in vastus lateralis in the orienteers were not significantly smaller than in controls. However the type I fibres in gastrocnemius was significantly ($p < 0.01$) smaller than the type I fibres in vastus lateralis of the controls (Table IV).

Activities of the oxidative enzymes SDH and HAD were higher in orienteers than in controls in both vastus lateralis and gastrocnemius (Table II). Both SDH and HAD activities



Fig. 1 Serial sections stained for myof brillar ATPase after preincubation at pH 10 3 (A), 4.3 (B) and 4.6 (C), NADH-dehydrogenase (D) and α-glycerophosphate dehydrogenase (E). 1 A–E are from vastus lateralis of a control subject and 2 A–E are from gastrocnemius of an orienteer Note the absence of IIB fibres (stains positively at pH 4 6 and negatively at 4.3) in gastrocnemius of the orienteer and the homogeneous staining intensity for NADH-dehydrogenase and α-glycerophosphate dehydrogenase in the orienteer compared t the control subject.

were higher in gastrocnemius than in vastus lateralis. There was no significant difference in SDH or PFK activity between the D and I group but the HAD activity was significantly higher in group D with the more extreme "distance" training (Fig. 4).

There was no significant difference between SDH activity in type I and II muscle fibres in gastrocnemius of the orienteers, *i.e* the ratio between SDH in type I and SDH in type II fibres was on the average 1 0 In vastus lateralis it was 1 3 In control subjects (Essén et al. 1975) it was 1.5 in both gastrocnemius and vastus lateralis (Table III).

The difference in SDH type I/SDH type II ratio between gastrocnemius of orienteers and controls is significant. There was no significant difference between group D and I with regard to the ratio SDH in type I/SDH in type II fibres. However among orienteers the highest individual ratio 2.2 was found in vastus lateralis of group I and the lowest one 0.9 in gastrocnemius of group D

Discussion

Fibre composition and fibre areas This investigation showed a difference in proportion type I fibres between leg muscles of the *orienteers* and leg muscles of the *controls*, but not between

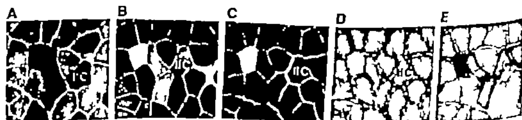
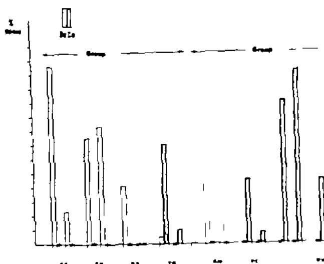


Fig. 2. Serial sections from vastus lateralis of an orienteer stained for myofibrillar ATPase after preincubation at pH 10 3 (A), 4.3 (B) and 4 6 (C), NADH-dehydrogenase (D) and α-glycerophosphate dehydrogenase (E). Note the occurrence of IIC f bres.



3 Percentage IIC and IIB fibres in vastus lateralis (V) and gastrocnemius (G) in the orienteers of group D and I. The bars represent individual values. The median value of the percentage IIC fibres in the control group 0 1 and in the orienteers 2 3

muscle and arm muscle within the group of orienteers (Table I). There were no biopsies taken from deltoideus in the control group in this study but other investigations have shown that in untrained men the mean percentage type I fibres in m. deltoideus is similar to that in vastus lateralis and gastrocnemius although there is a large interindividual variation (Gollnick *et al.* 1972, Johnson *et al.* 1973). The similarity between trained and untrained muscle within the group of orienteers suggests that the extreme endurance training has not caused the high percentage type I fibres. A plausible explanation then is that a high percent of type I fibres is an advantage in the performance of physical work of endurance character and may thereby act as a selection factor

In relation to IIB fibres leg muscles of the orienteers contained more IIA and IIC fibres than both leg muscles of the controls and arm muscles of the orienteers. This points at the probability of training induced alteration in the subgroup pattern. The training probably has produced a conversion of IIB to IIA fibres, which in general have higher oxidative

TABLE II Enzyme activities expressed as µmol [illegible] min of vastus lateralis and gastrocnemius of the elite orienteers and of vastus lateralis of the control subjects (Hedberg and Jansson 1976).

		SDH	HAD	PFK
Group D–I				
Vastus lateralis	n = 8	14 8 ± 2.7	12.8 ± 2.4	12 6 ± 0 7
Gastrocnemius	8	19 0 ± 1.1	15 5 ± 2 4	12 8 ± 1.6
Control group				
Vastus lateralis		10 4 ± 2.6 (n = 53)	10 4 ± 2.9 (n = 51)	15.7 ± 4.7 (n = 60)

Values are means [illegible] 0 05, p 0 01 p 0 001



Fig. 1 Serial sections stained for myofibrillar ATPase after preincubation at pH 10 3 (A), 4 3 (B) pH 4 6 (C), NADH-dehydrogenase (D) and α-glycerophosphate dehydrogenase (E). 1 A E are from vastus lateralis of a control subject and 2 A E are from gastrocnemius of an orienteer Note the absence of IIB fibres (stains positively at pH 4 6 and negatively at 4.3) in gastrocnemius of the orienteer and the homogeneous staining intensity for NADH-dehydrogenase and α-glycerophosphate dehydrogenase in the orienteer compared to the control subject.

were higher in gastrocnemius than in vastus lateralis. There was no significant difference in SDH or PFK activity between the D and I group but the HAD activity was significantly higher in group D with the more extreme "distance" training (Fig. 4).

There was no significant difference between SDH activity in type I and II muscle fibres in gastrocnemius of the orienteers, *i.e* the ratio between SDH in type I and SDH in type II fibres was on the average 1 0 In vastus lateralis it was 1.3 In control subjects (Essén *et al* 1975) it was 1.5 in both gastrocnemius and vastus lateralis (Table III).

The difference in SDH type I/SDH type II ratio between gastrocnemius of orienteers and controls is significant There was no significant difference between group D and I with regard to the ratio SDH in type I/SDH in type II fibres. However among orienteers the highest individual ratio 2.2 was found in vastus lateralis of group I and the lowest one 0.9 in gastrocnemius of group D

Discussion

Fibre composition and fibre areas This investigation showed a difference in proportion of type I fibres between leg muscles of the *orienteers* and leg muscles of the *controls*, but not between

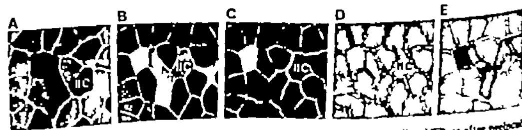


Fig. 2. Serial sections from vastus lateralis of an orienteer stained for myofibrillar ATPase after preincubation at pH 10.3 (A), 4.3 (B) and 4 6 (C), NADH-dehydrogenase (D) and α-glycerophosphate dehydrogenase (E). Note the occurrence of IIC fibres.

Table V Fibre areas of type I and type II muscle fibres in vastus lateralis, gastrocnemius and deltoideus of the orienteers and in vastus lateralis of the control subjects (Hedberg and Jansson 1976).

		Type I $\mu m^2 \cdot 10^{-2}$	Type II $\mu m^2 \cdot 10^{-2}$
D+I			
[illegible]ocnemius	−6	35.0 ± 6.0	42.9 ± 6.2
[illegible]us lateralis	7	40.4 ± 6.1	42.2 ± 11.8
[illegible]oideus	4	44.0 ± 11.0	60.1 ± 11.3
[illegible]ol group			
[illegible]us lateralis	−62	48.4 ± 12.0	52.7 + 14.6

[illegible] are mean ± SD of the individual mean values estimated from 20–25 fibres of each type. [illegible] denotes [illegible]

[illegible] range as the orienteers (n = 24) had the same low proportion IIC fibres as the rest [illegible]e control group and consequently differed significantly from the orienteers (p < 0.001). [illegible] IIC fibre shows similarities to a fibre type which exists in fetal skeletal muscle and which [illegible]so frequently found in muscle of the newborn (Riley 1973 Colling-Saltin, personal [illegible]munication). The staining of IIC fibres is positive after both preincubation at pH [illegible] and 4.3. Since mitochondrial ATPase shows the same alkaline and acid stability as the [illegible] fibres, the stain characteristics of this fibre type might depend on an interference with [illegible]ochondrial ATPase. Samaha and Yunis (1973) have shown that mitochondrial ATPase [illegible]he muscle of the newborn rat is four times more active than actomyosin ATPase in the [illegible]e muscle. In addition, Riley (1973) found that during regeneration of an injured muscle [illegible] fibres repeat the developing sequence which has been described during fetal life. The [illegible]nerating fibres pass a stage, where the myofibrillar ATPase stain is positive after both [illegible]line and acid preincubation. Thus, the IIC fibres occurring in the orienteers might be [illegible]ured muscle fibres, in which the regeneration process has started. Against this hypothesis [illegible]aks the fact that no necrotic fibres or fibres in the myoblast form which are seen during [illegible]generation and regeneration (Riley 1973) have been observed in the biopsies from the [illegible]enteers.

Another explanation of the high percentage IIC fibres in the leg muscles of the orienteers [illegible]ay be an increased rate of the denervation—reinnervation processes which normally occur [illegible]t a slow pace (Morris 1969). In this connection it may be pointed out that with increasing [illegible]ge the denervation–reinnervation rate is progressively increased and grouping of type I [illegible]bres develops (Jennekens *et al.* 1971). Furthermore, an increased occurrence of IIC fibres [illegible] intermediate fibres (as judged from the NADH-dehydrogenase stain) has been described [illegible]so in connection with collateral reinnervation and type grouping in neuromuscular disease, [illegible]nd this increase appears without the occurrence of fibres in the myoblast form as in re[illegible]generation processes (Morris 1970, Edström, personal communication).

The fibre areas in the leg muscles of the orienteers tended to be small, especially the type I [illegible]bres in gastrocnemius, which is in contrast to the findings of increasing area of type I fibres [illegible]th endurance training (Gollnick *et al.* 1973 Saltin *et al* 1976). However in these longi[illegible]tudinal training studies the subjects were training on the bicycle ergometer at a rather high

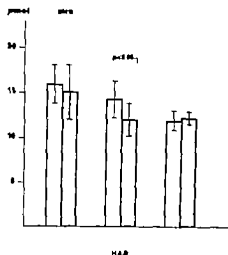


Fig. 4. Muscle enzyme activities in the ... bars represent the mean value ± SD of enzyme activity ... gastrocnemius and vastus lateralis of group D (4 subjects) and group I (4 subjects).

capacity and in the cat also have been found to be less fatiguable (Brook and Kaiser 1970 ... Jansson 1975 Burke *et al* 1973) Such a conversion might indicate that the training has altered the myosin molecule (cf Brooke and Kaiser 1970 b), which in turn might also have altered contraction characteristics. Support of this conversion hypothesis might be found in the fact that in the ATPase stain (after preincubation at pH 4 6) the colour intensity of different IIB fibres can vary from pale greyish to dark black in the orienteers, suggesting different stages of conversion. In the control subjects more distinct differences between IIA and IIB fibres were generally found. However it is not established if the basis of classification into IIA and IIB fibres depends on qualitative and/or quantitative differences in the characteristics of the myosin molecule of functional importance. Samaha and Yunis (1973) and Guth (1973) have reported that mitochondrial ATPase can interfere with the myofibrillar ATPase stain However the mitochondrial ATPase is both alkaline and acid stable and a mitochondrial suspension stains dark after preincubations at pH 10.4 as well as at pH 4.35 but both IIA and IIB fibres stain negatively at pH 4 35 This suggests that the difference between IIA and IIB is not dependent upon interference with mitochondrial ATPase.

IIC fibres occurred more frequently in the leg muscles of the orienteers than of the controls (Hedberg and Jansson 1976) This seems not to be directly an effect of the difference in type I–type II relationship since those control subjects with percentage type I fibres in the ...

TABLE III. SDH activity in type I and type II muscle fibres from vastus lateralis and gastrocnemius in ... orienteers.

	SDH in type I μmol × $g_{d.w.}$ min⁻¹	SDH in type II μmol × $g_{d.w.}$ min⁻¹	p	SDH type I / SDH type II
gastrocnemius	37.– ± 7.5 (36)	36.7 ± 7 1 (18)	> 0.05	1 0
vastus lateralis	23.7 ± 6.7 (34)	18.8 ± 8 3 (–8)	< 0 05	1.3

Values are means ± SD and values within parentheses are number of fibre pools analysed. Each pool contains 3–6 fibres of the same type (5–10 μg dry weight). $p < 0.05$ denotes that the difference between SDH in type I and type II fibres is significant at the level of 5 %.

is not significantly lower than in untrained subjects. Gastrocnemius also had higher ... SDH activity than vastus lateralis. Thus, the effect of training on SDH activity was ... pronounced in gastrocnemius. This is in good agreement with other studies which ha... e ... a greater involvement of gastrocnemius than of vastus lateralis during level running ... et al. 1974).

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relative load and the tension development in the leg muscles might have differed from the in the orienteers.

The number of capillaries has not been studied in all orienteers but in those studied mean $\pm SD$ of 4.6 ± 0.7 capillaries was found around each fibre, which does not differ from that found in untrained subjects (4.3 ± 0.8 in own unpublished normal material), but the number of capillaries per unit area in the orienteers was about 1.5–2 times normal. The capillary density in the untrained and trained subjects is in agreement with other studies (Andersen 1975, Brodal *et al.* 1976). That we did not find a high number of capillaries around each fibre, as other investigators have done, is mainly explained by the small fibre area in the orienteers. These findings regarding high capillary density and small fibre areas support the classic concept of the importance of short diffusion distance in the tissue (Krogh 1918) to facilitate oxygen transport to all parts of the muscle fibre during exercise.

Quantitative muscle enzyme measurements. Activities of the oxidative enzymes SDH and HAD were higher in the orienteers than in the controls. This could be an effect of training or the result of the difference in fibre composition between orienteers and control subjects. However, the subgroup of the controls mentioned above with the same fibre composition as the orienteers displayed the same levels of SDH and HAD activities as the whole control group. This suggests that the level of oxidative enzyme activities is dependent more on the degree of physical activity than on the fibre composition. Staudte *et al.* (1973) have found that sprint training in the rat increases enzymes in Krebs cycle but not the fat oxidizing enzyme HAD. This is probably related to the high rate of carbohydrate oxidation in this type of work. The present study suggests that long distance training increases the fat oxidation enzyme HAD approximately to the same extent as Krebs cycle enzyme. Furthermore, the HAD activity was highest in group D, the most extreme distance training group, although there was no difference in SDH or PFK activity between the groups. During prolonged heavy exercise plasma FFA increases to levels 2–3 times that at rest (Carlson *et al.* 1963) and consequently group D in all probability performs a great proportion of the training with high arterial FFA concentrations. Since arterial FFA concentration is the most important single determinant of FFA extraction by the working skeletal muscle (Hagenfeldt and Wahren 1971) the relative contribution of fatty acids to oxidative metabolism is in all probability high and this might be the factor which in turn leads to increased HAD activity.

In muscles of untrained subjects there are differences in staining intensity for e.g. NADH-dehydrogenase between type I and II fibres, and quantitative measurements of SDH in untrained muscles have shown a ratio between SDH in type I and SDH in type II fibres of 1.5 (Essén *et al.* 1975) (Fig. 4). The leg muscles of the orienteers showed a more homogeneous staining pattern for NADH-dehydrogenase suggesting that there are no or only minor differences in oxidative capacity (Fig. 1). In gastrocnemius also the quantitative measurements revealed that there was no significant difference between SDH in type I and SDH in type II fibres. Thus, the endurance training seems to have produced a marked increase in SDH activity especially in the type II fibres, which apparently have the ability to adapt to high oxidative demand. Thus adaptation might to some extent be the result of a conversion of IIB to IIA fibres. The ratio SDH in type I/SDH in type II in vastus lateralis on the other

...was not significantly lower than in untrained subjects. Gastrocnemius also had higher ...le SDH activity than vastus lateralis. Thus, the effect of training on SDH activity was ...ronounced in gastrocnemius. This is in good agreement with other studies which have ... a greater involvement of gastrocnemius than of vastus lateralis during level running ...ll et al. 1974).

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Acta physiol. scand. 1977. 100, 325–331

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Early Oestrogen-Induced Changes in Uterine Albumin Exchange in Mice

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Received 4 January 1977

Abstract

...dson, N. G. *Early oestrogen-induced changes in uterine albumin exchange in mice.* Acta physiol. scand. 1977. *100*. 325–331

...effect of the oestrogens, oestradiol and oestriol, on plasma albumin dynamics in the uterus was studied ...ice. A double isotope technique with ^{125}I- and ^{131}I-labelled human serum albumin was used. The opti... ...conditions to measure the extravascular accumulation of albumin were to use one labelled albumin ...permeability marker" (30 min circulation time) the other as plasma volume indicator (3 min circulation ...). The oestrogens increased the extravascular accumulation of labelled albumin in the uterus but not ...esophagus or heart muscle. The accumulation was maximal after 4 h (75 ... higher than in controls) and ...significant as early as 2 h after oestrogen administration. It cannot be decided whether the increase in ...that albumin accumulation was due to an increase in permeability and/or to an increase in the total ...face area of perfused capillaries.

...acute effect of oestrogens on the spayed uterus can be divided into two phases: firstly ...early response of increased blood flow, increased blood volume and oedema (Peterson ...& Spaziani 1969, 1971). This early vascular response is apparent shortly after injection. ...increased blood flow can be seen within 30–45 min in rats (*e.g.* Peterson and Spaziani ...69) and ewes (*e.g.* Huckabee *et al.* 1970, Killam *et al.* 1973). During this phase the wet ...eight of the uterus increases, but there is no increase in dry weight. Secondly there is an ...trophic phase beginning after 10–20 h with an increase in dry weight.

Oestriol and oestradiol are potent agents in inducing the early vascular response (Hisaw ...59). They have a high affinity to the oestrogen receptor but oestriol has a short retention ...me compared to oestradiol on the receptor site in uterus (Miller 1969) with much less ...bility to promote uterine growth.

The purpose of this study is to report a relatively uncomplicated quantitative method of ...timating oestrogen-induced effects on extravascular accumulation of albumin in the ...terus.

Some of these results were presented at the XVth Scandinavian Congress of Physiology ...nd Pharmacology, Århus 1976.

Materials and Methods

24–28 g mice of the NMRI-strain were used. They had free access to water and food (commer and a 12/12 h light/dark rhythm. All mice were spayed at least 8 days before priming with β–μg benzoate s.c. in 0.1 ml olive oil. The experiments were run 10 days after priming

To estimate permeability ^{125}I HSA (human serum albumin) (AB Atomenergi, Studsvik, Sw ^{131}I HSA (Radiochemical Centre, Amersham, England) were used. Immediately prior to the the iodine labelled albumins were purified by passage through a G-25 Sephadex column (Pharmacia, Uppsala, Sweden). Unlabelled bovine albumin (crystallized Sigma Chemical Co. Mo U.S.A.) was added so that the total amount of albumin injected was less than of own plasma albumin pool. The albumin was not screened (see further Sassen *et al.* 1968) belo of ^{125}I HSA was i jected into one retroorbital plexus and 50 μl of ^{131}I HSA was injected into the orbital plexus. Approximately 5 μCi of each radionuclide was injected.

30 s before death, under light ether anaesthesia, a 25 μl blood sample was drawn with a capi from that retroorbital plexus, where ^{125}I HSA had been injected (the isotope injected first) and in a Beckman 152 Microfuge. 10 μl of serum was counted for radioactivity. Serum or plas activities could be used tercha geably for practical reasons serum activities were counted calculating plasma albumin. The animals were killed by cervical fracture. The body was ope uterus immediately removed, dissected free from extraneous tissue, gently blotted and eighed balance (Sauter). Left ventricular heart muscle or diaphragm were used as control and treate The tissues were dried for at least 24 h at 100 to obtain dry weights. No effort as made plasma ^{125}I HSA concentration constant throughout the experiments. The activity of each counted in a 3-channel Packard gamma spectrometer. About 19% ^{131}I activity appeared in the and had to be corrected fo The statistical counting error was in no case above 2%. One can that a part of the jected ^{125}I and ^{131}I-labelled albumin was metabolized during the experime some free ^{125}I and ^{131}I-iodide would be present in blood and tissues. To determine the amoun and ^{131}I-iodide, heart muscle, liver kidney and uterus were pooled from the same animal. Th free isotopes in plasma was also determined. The tissues were homogenized and the prote cipit ted with cold 50% TCA after addition of one drop of 1 M 10 sodium ^{127}I-iodide to The activity of the precipitate and the supernatant was determined after centrifugation. The a radioactive iodide was less than 2%.

Oestriol (1,3,5(10)-oestratriene-3,16α,17β-triol) and oestradiol (1,3,5(10)-oestratriene-3,17β- Chemical Co.) were dissol ed in 50 propylene glycol in 0.9% NaCl and were given s.c. In control group received the appropriate vehicle. There were 4 5 animals in each group.

Technique in estimating xtravascular uterine uptak of albumin ^{125}I HSA was injected 10 before death. During this time fraction will penetrat the capillary wall and thus gi an extravascular uptak of albumi Obviously much of the activity will stay in the capillary lume a second injection was made 3 min before death with ^{131}I HSA. There are different opinions circulation time is optimal, if albumin is used as a plasma olume indicator (g Friedman 19 1971 Brody *et al.* 1974). However it has been shown that in mice there is no difference plasma volume whether 2 or 4 min are used (Sassen *et al.* 1968).

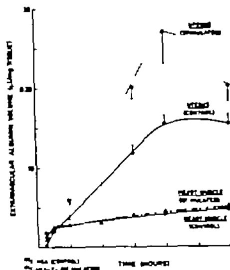
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$$\frac{^{125}\text{I HSA cpm/mg uterus}}{^{125}\text{I HSA cpm/}\mu\text{l plasma}} - \frac{^{131}\text{I HSA cpm/mg terus}}{^{131}\text{I HSA cpm/}\mu\text{l plasma}} \text{ gives } \frac{^{125}\text{I HSA cpm extravascularly/mg}}{^{125}\text{I HSA cpm/}\mu\text{l plasma}}$$

This numerical value is the "apparent Plasma Equivalent Extravascular Albumin Vol me (E tissue (Bill 1964, 1968).

Study of uterine total uptak of albumin at different ime after oestrogenic stimulation. The injected i.v. with ^{125}I-HSA 1 h before death nd w th ^{131}I HSA 30 min before death. This nec stimulated control groups for each oestrogen stimulation time. These recei ed the approp Here, diaphragm was used as control tissue. I these experiments the time-relationship afte stimulation could be followed. Oestradiol was given a dose of 0.1 μg.

Statistical significance was tested w th the Student's t test. A p-value below 0.01 was cons cant.



1. Effect of oestriol (E_3) stimulation time on (apparent Plasma Equivalent Extravascular Albumin Volume). ^{125}I-HSA and 0.1 µg E_3 or HSA and E_3 vehicle, respectively were injected at zero time. (Mean values are shown, vertical bars ±1 S.E.) *p < 0.05, **p < 0.01 ***p < 0.001

Results

It was important to know whether the kinetic behaviour of ^{125}I-HSA differed in any way between control and oestrogen treated animals. Plasma activity in the same control animal was measured after injection of ^{125}I HSA at zero time, at 1, 3, 5, 10 and 30 min. The same conditions were used for animals injected with 0.1 µg oestradiol 2.5 h before ^{125}I HSA injection. The samples were drawn from the uninjected orbit. The plasma activity of ^{125}I HSA in 3 control and 3 oestrogen stimulated animals, in percent of the plasma activity at 1 min, declined very much in parallel. At 30 min the values were 84.8, 74.5 and 86.4% versus 75.3, 75 and 79.8%, respectively. Thus, oestrogen did not alter the fate of plasma protein concentration appreciably.

The relation between stimulation time and vascular response is illustrated in Fig. 1. Here 0.1 µg oestriol and ^{125}I-HSA were injected simultaneously at zero time and the EAV was followed at 10 and 30 min, 1 h, 2 h, 3 h, 4 h and 6 h. ^{131}I-HSA was injected 3 min before death at each time investigated. Control animals received ^{125}I HSA only at zero time. There was a rapid uterine accumulation of ^{125}I-HSA both in control and oestriol-treated uteri which was far above that in heart muscle. Oestriol showed a tendency to increase uterine albumin accumulation after 1 h which was significant after 2 h of stimulation. The effect reached a maximum at 4 h and then declined.

Fig. 2 shows a slightly different way to analyze the relation between stimulation time and vascular response. Here the animals were stimulated by 1 µg oestradiol at zero time and a fixed circulation time of 30 min for ^{125}I-HSA and 3 min for ^{131}I HSA was used. The extravascular activity of ^{125}I-HSA was calculated as above. A maximum value is reached at 4 h with a decline towards 6 h.

Fig. 3 shows another experimental approach to the study of the time-response relationships. Here, fixed circulation times, 0.5 and 1 h, were used for the two labelled albumines

Materials and Methods

24–28 g mice of the NMRI strain were used They had free access to water and food (commerc and a 12/12 h light/dark rhythm. All mice were spayed at least 8 days before priming with 8 μg benzoate s.c. in 0.1 ml olive oil. The experiments were run 10 days after priming

To estimate permeability ^{125}I HSA (human serum albumin) (AB Atomenergi, Studsvik, Sw ^{131}I HSA (Radiochemical Centre, Amersham England) were used. Immediately prior to the ex the iodine labelled albumins were purified by passage through a G 25 Sephadex column in 0 (Pharmacia, Uppsala, Sweden) Unlabelled bovine albumin (crystallized Sigma Chemical Co Mo U.S.A.) was added so that the total amount of albumin injected was less than 2 of the own plasma albumin pool. The albumin was not screened (see further Sassen *et al.* 1968) below of ^{125}I HSA was injected i to one retroorbital plexus and 50 μl of ^{131}I HSA was injected into the o orbital plexus. Approximately 5 μCi of each radionuclide was injected.

30 s before death, under light ether anaesthesia, a 25 μl blood sample was drawn ith a capill from that retroorbital plexus, where ^{125}I HSA had been injected (the isotope injected first) and in a Beckman 152 Microfuge 10 μl of serum was counted for radioactivity Serum or plasm activities could be used interchangeably for practical reasons serum activities were counted an calculating "plasma" albumin The animals were killed by cervical fracture. The body was open uterus immediately removed, dissected free from extraneous tissue, gently blotted and eighed o balance (Sauter). Left ventricular heart muscle or diaphragm were used as control and treated l The tissues were dried f r at least 24 h at 100° to obtain dry weights. No effort was made t plasma ^{125}I HSA concentration consta t thro ghout the experiments. The activity of each ti counted in a 3-channel Packard gamma spectrometer About 19 % ^{131}I activity appeared in the and had t be corrected for The statistical counting error was in no case above 2 %. One cannot that part of the injected ^{125}I and ^{131}I-labelled albumin was metabolized during the experimen some free ^{125}I and ^{131}I iodide would be present n blood nd tissues. To determine the amount and ^{131}I iodide, heart muscle, liver kidney a d uterus were pooled from the same animal. The free isot pes in plasma was also determined The tissues were homogenized and the proteins cipitated with cold 50 % TCA after add tion of one drop of 1 M 10^{-4} sodium ^{125}I-iodide to ca The activity of the precipitat and the supernatant was determined after centrifugation. The amo radioactive iodide was less than 2 %.

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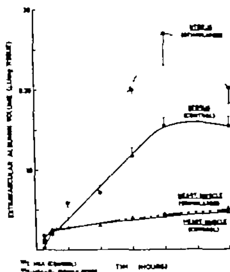
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$$\frac{^{125}\text{I HSA cpm/mg uterus}}{^{125}\text{I HSA cpm/}\mu\text{l plasma}} - \frac{^{131}\text{I HSA cpm/mg uterus}}{^{131}\text{I HSA cpm/}\mu\text{l plasma}} \text{ gives } \frac{^{125}\text{I HSA cpm extravascularly/mg ute}}{^{125}\text{I HSA cpm/}\mu\text{l plasma}}$$

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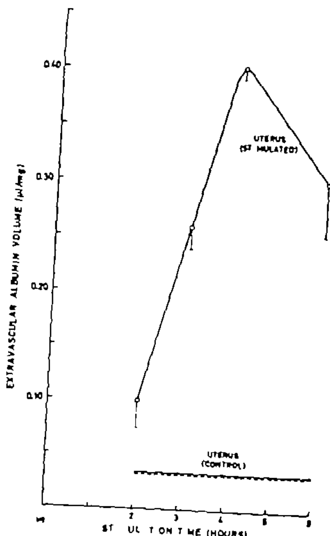


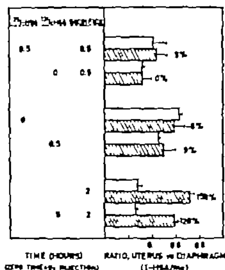
Fig. 4. Effect of different oe stimulation times on EA\ jected at zero time. A fixed time (0.5 h) for ¹³¹I-HSA was further legends to Fig 1.)

In the same animal. Oestradiol 0 1 µg or vehicle were injected at zero time. Uterine accumulation was related to diaphragm accumulation Also in this experimental clear-cut oestrogen-induced increase in uterine albumin accumulation is observed (values calculated in per cent of controls because of the above mentioned different ci times for ¹³¹I HSA and ¹²⁵I HSA)

Discussion

The characteristics of the microcirculation differ from tissue to tissue. This was sugg Clark and Clark (1935) and Grotte (1956) and is now well established (e.g. Majn One characteristic of the uterine vascular bed is its sensitivity to oestrogenic stim The basis for this specific sensitivity is not yet known we have focused our stu vascular permeability

Many different methods are available for estimating permeability In recent years l proteins, e.g albumin, have become increasingly used in permeability studies. Mos only one labelled protein has been used. The use of macromole ..d belled with d



[illegible] Effect of different stimulation times with [illegible] (E₂) on albumin accumulation in uterus [illegible] ^{125}I-HSA was injected into the same [illegible] 1 h and ^{131}I-HSA 0.5 h before sacrifice. E₂ [illegible] was injected at zero time. Mean values are [illegible], vertical bars represent 1 S.E. and figures [illegible] of the corresponding control. Significance [illegible] reached after 2 h stimulation time.

[illegible]nuclides (here ^{125}I- and ^{131}I-albumin) has obvious advantages. The double isotope [illegible] as used here eliminates possible interindividual differences in organ plasma [illegible]—and hence makes it possible to subtract the amount of the permeability marker [illegible] in the vessel lumen.

[illegible] number of studies were performed to find out optimal conditions for the use of these [illegible] to study the effect of oestrogens. Circulation times between 10 min and 6 h for [illegible] permeability marker (^{131}I-HSA) were tested. Finally a short circulation time (30 min) [illegible] found to be the most suitable. For instance, it was found that the decline in plasma [illegible] is practically negligible during a 30 min circulation time. Circulation times of [illegible] h, give lower estimates of the EAV increase, mainly because of the rapid EAV in[illegible] also in controls. This is illustrated in Fig. 1 and Fig. 2. If a variable circulation and [illegible] time is used, as in Fig. 1 the ratio EAV stimulated animals/EAV control [illegible] is about 1.6, 1.7 and 1.8 after 2, 3 and 4 h, respectively. If a fixed circulation time [illegible] 30 min is used (Fig. 2) sensitivity is increased since the ratio will be more than 3, 8 and [illegible] after 2, 3 and 4 h of stimulation time, respectively. In a separate experiment (not [illegible]) a fixed stimulation time for oestradiol (? h) and a variable circulation time for [illegible]I-HSA was used. The ratio was 3.0 after 30 min, [illegible]1 after 2 h and 2.0 after 4 h circula[illegible] time, re-emphasizing the advantage of a comparatively short circulation time. The [illegible] changing effect of oestrogen affecting the vascular system and surrounding [illegible] is a problem which further strengthens the importance to define a suitable circulation [illegible].

The conventional way (Kalman 1955) to measure oestrogen stimulated accumulation of [illegible] albumin in the uterus has been to use only one radionuclide labelled albumin and an [illegible] ratio (e.g. uterus/diaphragm). This approach is shown in Fig. 3. However much of [illegible] activity will be located intravascularly (Davis 1960). It is therefore not possible to measure [illegible] amount of labelled extravascular albumin in a single animal. It is of course possible to

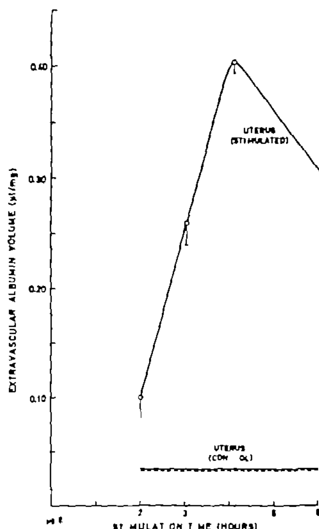


Fig. 2. Effect of different oestradiol stimulation times on EAV E_2 wa jected at zero time. A fixed circ time (0.5 h) for ¹²⁵I-HSA was used (further legends to Fig 1)

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use two groups of animals and different labelled albumin circulation times. If, on the other hand, one finds it unnecessary to use one radionuclide albumin as a plasma volume indicator, this isotope can be used as shown in Fig. 3 Here, the increasing accumulation of labelled albumin from zero time after oestrogenic stimulation and onwards is observed in the same animal, *e.g* at 0 5 and 11 h and thus we get an idea of what time relations are interesting from a vascular point of view

From Fig. 1–3 it can be clearly seen that both oestriol and oestradiol rapidly increase the accumulation of labelled albumin in the uterus. Fig. 1 and 2 show that after approximately 2 h stimulation, there is a large increase in labelled albumin accumulation reaching a maximum at 4 h. The effect is of course dose-dependent and the doses used here are near maximal for oestriol and supermaximal for oestradiol (unpublished). Fig. 1 shows that there is a rapid increase in uterine EAV in controls. The decline in EAV from 4 to 6 h in stimulated animals is most probably due to the vanishing stimulatory effect of oestriol, and to a "self inhibitory" effect of the oedema itself and is not observed in controls.

The mechanism for an increased extravascular accumulation of a solute is difficult to determine It might be due to an increase in permeability or to an increase in the total surface area of perfused capillaries, as termed by Renkin (1959 1965) the permeability surface product, PS

Hechter *et al.* (1941 1942) were the first to claim the presence of an increase in the capillary permeability after oestrogenic stimulation. Several later investigations seem to support this view (reviewed by Szego and Roberts 1953 and Spaziani 1975, *e g* Kalman, Kalman and Lowenstein 1958 Davis 1960, Cecil *et al* 1966, Spaziani and Szego, Peterson and Spaziani 1969 1971 Ham *et al* 1970). However a critical review of publications does not rule out the possibility that what is observed as increased permeability is mainly due to an increase in the total surface area of perfused capillaries. The situation concerning uterus and oestrogenic stimulation might be analogous to the situation of hyperemia in skeletal muscle in activity (Folkow and Mellander 1970 Radawski *et al.*

Further work is necessary to define which factor in the PS-product of the uterus undergoes the largest change after oestrogenic stimulation.

I am indebted t Dr L. Terenius and Prof A Bill for valuable advice The work as supported by the Swedish Cancer Society (Grant no 43-B75-09X to L. Teren us) and by the Medical Faculty of the University and the Svenska Läkaresällskapet.

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il equivalent of the pore, Perl (1971) concluded that the constricted pore effect largely ...fed out the effect of the osmotic reflection coefficient leading to maintenance of the ...al 37 Å slit width estimate.

...e "indicator diffusion" method (Crone 1963 a) for study of transcapillary transport is ...l on solute diffusion. Consequently it is not complicated by osmotic reflection coeffi... corrections. Applying this technique Crone (1963 b) found absence of diffusion restric... to inulin as compared to sucrose across capillary membranes in skeletal muscle. A ...finding, this led to questioning of the Pappenheimer 30 Å pore as absence of ...ted diffusion could only be accounted for by assuming existence of larger pores func... ...ng as a non-restricting continuous layer interposed somewhere between blood and ...stitial fluid. With a similar approach, Alvarez and Yudilevich (1969) obtained results ...ting absence of restricted diffusion in heart muscle capillaries in contrast to results ...ted by Schafer and Johnson (1964). In human skeletal muscle Trap-Jensen and Lassen ...) found evidence of pronounced restriction to inulin as compared to ^{51}Cr EDTA (^{51}Cr ...ene-diamine-tetraacetate).

...cutaneous tissue, the capillary diffusion capacity CDC (the permeability–surface area ...uct of Renkin, PS) of the monovalent chelate anion ^{51}Cr EDTA was determined in a ...ous study (Paaske 1976) with the single injection, external registration method which is ...d on indicator diffusion (Sejrsen 1970). In the present study CDC for ^{57}Co-cyan...balamin (^{57}Co-B12) has been determined with the same preparation and technique ...the aim of elucidating whether this substance, as compared to ^{51}Cr EDTA, is subject ...tricted diffusion during the passage across the continuous endothelium of cu...ous capillaries (Bennett, Luft and Hampton 1959 Majno 1965).

Experimental procedure

...taneous tissue preparation ...as made in accordance with techniques described previously (Paask 1976): ...ments were performed on female rabbits of ...bite Danish country breed, which had been fasting for ... Anesthesia was induced with sodium pentobarbitone 25 mg/kg I... and supplementary doses were ...s required. Rectal temperature was kept constant at 38°C. Tracheotomy was made and an endo...tracheal tube was introduced. Blood pressures in the right atrium and the common carotid artery were ...ured via polyethylene catheters, Statham strain-gauge transducers and an oscilloscope. Tracings were ...rded on ... Varian ...rter. Through ... median section the left abdominal wall was bluntly dissected in the ...layer of loose connective tissue. The right common iliac artery was ligated and cut 1 cm distal to the ...bifurcation, and the aorta was clamped. The needle (external diameter 0.4 mm) of ... micro-syringe ...introduced upstream into the right common iliac artery. The clamp on the aorta was removed, the ...al was heparinized, and hematocrit value was determined. A rubber band was placed around the left ...ound circumflex iliac artery and led through ... polyethylene tube. Two minor branches from the left ...ound circumflex iliac artery to abdominal wall muscles and inguinal fat pad were ligated. The loosened ...s pulled laterally and the exposed surface was covered ...th gauze and plastic sheets. The skin ...as ...a constant temperature of 37°C by t... thermostatted copper plate heating the hairy surface. A one inch ...(Tl) scintillation detector (PW 4111, Type A, Philips, the Netherlands) was collimated to see the skin ...ortion only and connected to ... universal counting gamma spectrometer (Medrtronik a/s, Denmark) ...sed around the 0.122 MeV photopeak of ^{57}Co. Papaverine in isotonic saline was administered locally ...he inside of the skin and on the outside of the left profound circumflex iliac artery at the aortic bifurca... 0.5 ml (20 mg/ml) papaverine ...as injected into the left profound circumflex iliac artery over ... period ...min. 150 µg (30 µg/ml) unlabelled cyanocobalamin was injected via the catheter in the right atrium. ...(300 µCi) ... Co-B12 (specific activity 150–300 µCi/µg cyanocobalamin; The Radiochemical Centre Ltd ...sham, Great Britain ... with the following technique: The tip of the needle was introduced

Acta physiol. scand. 1977 100 332–339
From the Institute of Medical Physiology B, University of Copenhagen, Denmark

Absence of Restricted Diffusion in Cutaneous Capillaries

By

WILLIAM P PAASKE

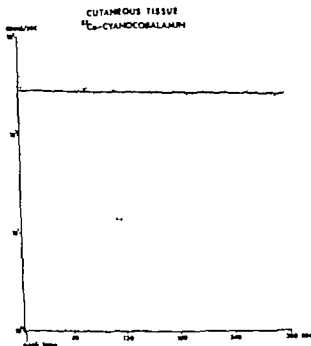
Received 6 January 1977

Abstract

PAASKE, W P *Absence of restricted diffusion in cutaneous capillaries.* Acta physiol. s 1977 *100* 332–339

Capillary permeability in cutaneous tissue fo ^{57}Co-cyanocobalamin (^{57}Co-B12) as determined single i jection, external registration method. The capillary diffusion capacity CDC (the permeab surface area product, PS) was ..3 ml/100 g min. Capillary extraction was 0.48 at a plasma flow of 100 g min Results were compared to ^{51}Cr EDTA data from a previous study with identical meth preparation. As CDC(^{51}Cr EDTA)/CDC(^{57}Co-B12) was 1 61 and as D(^{51}Cr-EDTA)/D(^{57}Co-B12 ratio between the free d ffusion coefficient in water at 37°C, was 1 79 it is concluded that restricted di does not occur in cutaneous tissue for ^{57}Co-B12 as compared to ^{51}Cr EDTA, i.e. ^{51}Cr EDTA and ^{57}C diffuse across the capillary membrane of cutaneous tissue at rates proportional t their respect diffusion coefficients in water The Pappenheimer equivalent pore radius estimate of 30 Å and the Ka sky interendothelial 40 Å slit width are both defective in explaining the experimental data. The tran thelial patent channel system of fused vesicles (Simionescu, Simionescu and Palade 1975) is a p structural equivalent for the present f ndings. The results support the hypothesis that capillaries of tinuous type exhibit similar permeation characteristics regardless of the tissue in hich they are lo

An array of equivalent aqueous pores was suggested by Pappenheimer Renkin and Bo (1951) as an operational term for describing a physical property of the capillary blo tissue barrier This pathway model for transmembraneous permeation of lipid-insoluble utes was based on measurements of transcapillary flux at zero filtration applying the iso metric "osmotic transient" method to the cat hindlimb preparation. As the permea coefficients for a series of indicators decreased more than could be anticipated from decrease in the free diffusion coefficients the restricted diffusion theory (Faxén 1922, F 1936, Pappenheimer *et al* 1951 for survey see Landis and Pappenheimer 1963) was f applicable to the experimental data. The original estimate of a circular cylindrical po 30 Å radius or alternatively a slit width of 37 Å was shown to be defective (Ussing 1 Grim 1953 Kedem and Katchalsky 1958) as account had not been taken of the influenc the osmotic reflection coefficient on osmotic pressure (Staverman 1951). "Correction the Pappenheimer data with calculated reflection coefficients leads to an equivalent p size estimate in the order of 140 Å (Michel 1972) However from an elaborate mathema analysis based on the Karnovsky (1967 1968) interendoth



1 Experimental curve (Exp. no 4). The dots represent the recorded count values corrected for back... activity. The monoexponential regression line was calculated by the "least square" method in the interval from 150 to 300. The intravascular transit curve (crosses) was obtained by subtraction of ... line from the original count values (dots). For details, see text

... in a semilogarithmic diagram. At time zero the bolus injection of 57Co-B12 was given. ... maximum count value of 683 cps was reached at 3rd s. As computed in the time interval ... 150 s to 300 s after bolus injection the slope of the regression line was 0.000528 s^{-1} ... a standard deviation of 0.000118 s^{-1} giving a value of 286 at peak time. The capillary ...action became (286/683) 0.419 The mean transit time of tracer in plasma was 0.405 ... which led to a plasma flow of f(pl) = (1/0.405) (1 0.399) 0.03 100 (ml/100 g min) = ...5 ml/100 g min. Consequently CDC 4.45 0.94 ln (1 0.419) (ml/100 g min) = 2.3 ...100 g min.

...TABLE II Some physical properties of 57Co-cyanocobalamin (57Co-B12) and 14C-ethylene-diamine-tetra-acetate (14C-EDTA). CDC(14C EDTA)/CDC(57Co-B12) for cutaneous tissue is given for comparison

	14C EDTA		57Co-B12
...lecular weight	341.2		1353.5
...efficient ...	0.700 10^{-5}		0.390 10^{-5}
(Å)	4.7		8.4
(Å)	5.1		8.9
D(14C EDTA)/D(57Co-B12)		1.79	
CDC(14C EDTA)/CDC(57Co-B12)		1.61	

TABLE I Capillary extraction, E, and capillary diffusion capacity CDC, of [57]Co-cyanocobalamin a cutaneous tissue P_d is the permeabil ty coefficient as calculated from CDC and an assumed capil surface area of 70 cm /g. $\bar{x}$ is mean value and S.E. denotes standard error of the mean

Exp No	E per cent	CDC ml/100 g min	P_d 10^6 cm/s	Plasma flow ml/100 g min	Blood flow ml/100 g min	Hct fraction	Peak time s
1	31.2	2.2	0.52	6.2	10 0	0 38	3
	55..	1 7	0.40	..3	3.6	0.36	3
3	50 5	2.3	0.55	3.5	5 6	0.37	2
4	41 9	2.3	0.55	4.5	7.5	0 40	3
5	60 1	2 4	0.57	..8	4 4	0.36	
6	47.2	3 1	0.74	5 1	8.2	0 38	4
$\bar{x}$	47 7	2.3	0.56	4 1	6 6	0.38	3
S.E.	4.2	0..	0 04	0 6	1 0	0 01	1

into the left profound circumflex iliac artery and the rubber band was pulled to press the arterial wall against the needle. The bolus injection was given, compression was terminated, and the needle was drawn and removed The whole injection procedure had a duration of about ~2.5 s. Activity was printed out by the spectrometer each s without t me loss. Via an interface u lt the spectrometer was connected to a tape puncher (Typo 4070, AB Facit Sweden) providing a paper tape used for computer input. Activity was recorded for 300 s. Counts remaining n the field after removal of the skin was measured and used as background which was subtracted from the recorded count values.

Calculations

The recorded curve function was computer analysed as described earlier (Paaske and Nielsen 1976). The count value of the i jected dose of [57]Co-B1 was taken as the highest recorded value and peak time as the time t which this alue occurred The part of the curve recorded from 150 s to 300 s was monoexponentially extrapolated to peak t me and subtracted from the riginal curve to give the intra ascular transit function. Intravascular mean transit time was computed as $\bar{t}(iv)$ = area/height (Zierler 1965). Plasma flow was calculated as f(pl) = ($1/\bar{t}$( )) λ 100 (ml/100 g min) (Kety 1951). λ denotes intra ascular plasma space which was estimated at 0.03 (1 − Hct) (ml/g) where Hct is hematocrit value (Paaske 1976). The value of the monoexponentially extrapolated curve at peak time expressed as a fraction of maximum count value gives the capillary extraction, E. The CDC def ned as the unidirectional fl of permeable tracer across the capillary membrane per 100 g tissue per un t concentration d fference cross the capillary (Lassen and Trap-Jensen 1968 b) was calculated as CDC = − f(pl) K ln (1 − E)(ml/100 g min) where K is a constant for conversion ml of plasma to ml of plasma ultraf ltrate A K-value of 0 94 was used (Lassen and Trap-Jensen 19..

Results

The experimental results are presented in Table I Mean value of capillary extraction was 0 477 with a standard error of the mean S E. of 0 042 in 6 expts. The mean capillary diffusion capacity CDC was 2.3 ml/100 g min (S E. 0.2 ml/100 g min) Assuming a capillary surface area in cutaneous tissue similar to that in resting skeletal muscle of 70 cm /g (Pappenheimer *et al* 1951) a mean permeability coefficient P_d of 0.56 10^{-6} cm/s (S.E. 0.04 10^{-6} cm/s) was obtained The plasma flow was 4 1 ml/100 g min (S E. 0 6 ml/100 g min) corresponding to a blood flow of 6 6 ml/100 g min (S E. 1 0 ml/100 g min) at a hematocrit value of 0.38 (S.E. 0.01). The mean peak time was 3 s (S E. 1 s).

An experimental tracing (Expt no 4) is shown in Fig. 1 The computer printout presents the recorded count values corrected for background activity (do tted as function

IV Calculated equivalent pore radius (P) or slit width (S) in tissues with continuous capillaries. RD and RD denote presence and absence of restricted diffusion for inulin or ^{57}Co-B12 as compared to sucrose or ^{51}Cr EDTA

	Pore radius or slit width Å	Reference
[illegible] tissue	RD	Paaske 1977 (present study)
[illegible]al muscle	P 30, S 37	Pappenheimer Renkin and Borrero 1951 Perl 1971
	P 35–45	Grotte 1956
	P 30–45	*Renkin and Pappenheimer 1957*
	RD	Crone 1963 b
	P 40–45	Landis and Pappenheimer 1963
	S 40	Trap-Jensen and Lassen 1971
	P 31 39	Dunn, Long and Yao 1977
	– RD	Paaske 1977 b
	RD	Paaske and Sejrsen 1977
[illegible] tissue	RD	Paaske 1977
	P 90–150	Boyd *et al.* 1969
	P 30–45	Wangensteen, Wittmers and Johnson 1969
	P 40–50	Taylor and Gaar 1970
	P 150	Normand *et al.* 1971
	RD	Schafer and Johnson 1964
	P 35	Vargas and Johnson 1964
	P > 80–100	Alvarez and Yudilevich 1969
	S 90–100	Bassingthwaighte, Yipintsoi and Grabowski 1975

the findings of the present study are comparable to data obtained from other tissues with similar capillary morphology. Table III presents permeability coefficients, P, for ...-B12 in tissues with capillaries of continuous type. Also, the ratios CDC(C EDTA)/ C(^{57}Co-B12) are given. The data support the hypothesis suggested earlier (Paaske 1976) ... capillaries of the continuous type exhibit similar permeation characteristics regardless of the tissue in which they are located.

As the CDC ratio values mentioned above are not commensurate with restricted diffusion they do obviously not allow calculation of an equivalent pore radius. They also seem incompatible with the belief that transport occurs through Karnovsky tight junctions, even considering that the width has recently been suggested to be about 60 Å (Casley-Smith 1976) rather than 40 Å as originally estimated (Karnovsky 1967 1968).

Electron micrographic search for the structural equivalents of the physiological transport pathways has been pursued vigorously but it is well worth emphasizing with respect to the pore theory that "the assumption is nowhere made that all the pores are uniform and circular, that they all have the same radius, or that they remain fixed in position or time. Indeed, the equivalent pore radius need not be the actual pore radius" (Solomon 1968). Recently however Simionescu *et al.* (1975) succeeded in demonstrating by electron microscopy that ...e peptide tracers with a molecular radius corresponding to that of ^{57}Co-B12 (about 10 Å) did not pass the skeletal muscle capillary blood to tissue barrier *via* tight junctions but *via* vesicles. The vesicles where either isolated or fused to form patent channels across the endothelial cell. The authors conclude that the channels appear *to be the most likely candidates* for structural equivalents of the aqueous channel system but point out that further work remains to establish the significance of their findings. A similar channel system has also

TABLE III Permeability coefficient, P_d for ^{57}Co-B12 and the ratio CDC(^{51}Cr EDTA)/CDC in tissues with continuous capillaries. The capillary surface area as assumed to be 7 cutaneous tissue (Paaske 1976) and in skeletal muscle (Pappenheimer Renkin and Bor and 35 cm^2/g in adipose tissue (Gersh and Still 1945) The ratio between the free di efficients in water at 37°C, D(^{51}Cr EDTA)/D(^{57}Co-B12), is 1 79 (see Table II).

Tissue	P_d 10^5 cm/s	CDC(^{51}Cr EDTA)/ CDC(^{57}Co-B12)	Method	Reference
Cutaneous tissue	0.56	1 61	SI	Paaske 1977 (present
Adipose tissue	0.52	1.82	SI	Paaske 1977 a
Skeletal muscle	0 40	2.00	TC	Lassen and Trap-Jen
	0 41	1 79	SI	Paaske 1977 b

Abbreviations SI si gle injection, external counting method TC: tissue clearance method.

Discussion

The method and its application to the present preparation have been discussed in previous study (Paaske 1976)

The molecular weight of ^{57}Co-B12 is 1353 5 (Table II) and the free diffusion in water at 37°C of the preparation employed in the present study D(^{5} Co-B12), mined to be 0.390 10^{-5} cm /s (S E. 0.004 10^{-5} cm /s, n = 5) using a technique d in agar at 37°C (Sejrsen 1976 personal communication) This corresponds to Einstein molecular radius, a_{S-E}, of 8 4 Å and a molecular radius, a_e, of 8.9 Å when from the formula of empirical viscometric correction, $a_e = a_{S-E}(1 + (0.5/a_e))$ (S Solomon 1961) D(^{57}Co-B12) = 0 390 10^{-5} cm /s is some 10 per cent higher than 10^{-5} cm /s estimate from the equation $D\sqrt{MW}$ = constant where MW is molecul As thus formula can be defective in estimating D for molecules with MW ~1 000 mentally determined D(^{5} Co-B12) was used in the calculations below The D determinations presented evidence that the indicator preparation used in this monomeric to at least the 0 001 level of the total dose (Sejrsen 1976, pers munication) Although the Co-B12 molecule cannot be regarded as inert as bound to receptors on plasma proteins and cells (Lassen and Trap-Jensen 196 binding should be negligible due to the injection of unlabelled cyanocobalamin p injection of the radionucleide.

In a previous study on cutaneous tissue performed with experimental techniqu present series the average value of CDC(^{1}Cr EDTA) was found to be 3 7 ml/10 an average capillary extraction of 0.59 and a plasma flow of 4 8 ml/100 g min (Pa Some physical constants of ^{1}Cr EDTA are presented in Table II

The ratio D(^{51}Cr EDTA)/D^5 (Co-B12) = 0 700 10^{-5} (cm /s)/0.390 10^{-5} (cm /s one or both substances were subject to restricted diffusion in cutaneous tissu CDC(^{51}Cr EDTA)/CDC(Co-B12) should be larger than 1 79 However 3 7 (ml/1 2.3 (ml/100 g min) = 1 61 for which reason it is concluded that restricted diffusio exist across capillary blood to tissue membranes in cutaneous tissue for ^{57}Co-B1 pared to ^{1}Cr EDTA.

The literature provides no data regarding restricted diffusion in cutaneous tis

V Calculated equivalent pore radius (P) or slit width (S) in tissues with continuous capillaries. RD and RD denote presence and absence of restricted diffusion for inulin or [57]Co-B12 as compared to sucrose or [51]Cr-EDTA.

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	P 31–39	Durn, Long and Yao 1972
	RD	Paaske 1977 b
	RD	Paaske and Sejrsen 1977
us tissue	RD	Paaske 1977
	P 90–150	Boyd et al. 1969
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	P 40–58	Taylor and Gaar 1970
	P 150	Normand et al. 1971
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As the CDC ratio values mentioned above are not commensurate with restricted diffusion do obviously not allow calculation of an equivalent pore radius. They also seem incompatible with the belief that transport occurs through Karnovsky tight junctions, even considering that the width has recently been suggested to be about 60 Å (Casley-Smith 1976) rather than 40 Å as originally estimated (Karnovsky 1967 1968).

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been claimed present in endothelial cells of another continuous capillary rich in adipose tissue (Blanchette Mackie and Scow 1971) and it might prove to be a universal feature of the continuous capillary

Recent physiological estimates of equivalent pore size have shown a tendency towards increasing pore radius (Table IV) and there is increasing evidence that the passage of lipid-insoluble solutes across the continuous capillary membrane may not occur through the Karnovsky slits. The patent channel vesicular system, however is in concert with findings of a number of physiological studies (Kruhoffer 1946, Crone 1963 b, Alvarez and Yudilevich 1969 Boyd *et al* 1969 Normand *et al* 1971 Bassingthwaighte, Yipintsoi and Grabowsky 1975 Paaske 1977 a and b Paaske and Sejrsen 1977)

It is concluded that restricted diffusion for ^{57}Co-B12 as compared to ^{51}Cr-EDTA does not occur in cutaneous tissue capillaries. The original Pappenheimer equivalent pore radius estimate and the Karnovsky slit are both defective in explaining the experimental data. The intraendothelial patent channel system of fused vesicles is a possible structural equivalent for the physiological findings. The hypothesis is supported that capillaries of continuous type exhibit similar permeation characteristics regardless of the tissue in which they are located

Dr Per Sejrsen is gratefully acknowledged for discussions, criticism of the manuscript and for having performed determinations of the free diffusion coefficients of the indicators.

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the water economy in animals. In the present work, these kidney functions were studied — to provide an insight into the adaptive responses of reindeer to environmental ct.

Material and methods

and care. The study was done in September and October when the reindeer had been indoors one month after summer pasture. The experiments were performed in metabolic stalls with room temperature of 20±1 C, under laboratory conditions to which the animals, two non-pregnant female (b.wt. 44 kg), had been previously well adapted. In order to avoid the effects of diets deficient in — the animals were fed twice daily a ration, the crude protein content of the dry matter being about — consisted of cut grass, commercial pelleted ration and grain meal with free access to water. The sodium balance of the reindeer was maintained by addition of a mineral mixture containing 10 g daily. The sampling was started in the morning before the animals were fed and no food was given during the experiments.

Hydration and dehydration. Hydration was performed with a water load of 6.5 litres (10 % of body weight) introduced through a stomach tube into the rumen, dehydration by not giving the animals any water to drink for 48 h.

Blood samples and injections. Blood samples were taken from the jugular vein. In order not to disturb the animal the intravenous injections of exogenous ADH (Pitressin "Parke-Davis") were made through a permanent venous catheter.

Urine collection and analyses. The urine was collected in 5 or 10 min samples via a retention catheter. "—" osmometer was used for determination of urine and plasma osmolality. Plasma osmolality of — mosm/kg was used for calculations of the renal free water clearance (C_{H_2O}). Urine [Na^+] and [K^+] determined by using flame photometry, the urine and plasma urea by liberating ammonia with urease (— and Marbach 1962). In addition to plasma osmolality the plasma protein concentration determined by the Biuret method, was used to estimate the state of dehydration (Kutscher 1971). Glomerular filtration rates were estimated by the endogenous creatinine clearance method. Blood and urine creatinine analysed by "Technicon" autoanalyser.

Results

Renal responses to water loading. Repeated experiments were performed in both reindeer. The diuresis began within 30 min after administration of water, the peak lasting for only about 40 min, was reached very quickly (renal C_{H_2O} 24±5 ml/min S.E.). Thereafter the urine flow rapidly declined but C_{H_2O} remained positive for several hours (Fig. 1).

Sodium excretion was high already during antidiuresis and did not increase during the development of water diuresis. The increase of potassium and urea excretion during increasing urine flow was slight. The excretion rate of all three solutes decreased somewhat during declining urine flow rate (Fig. 1).

Responses to water restriction. The second day of water deprivation food consumption was markedly reduced and the reindeer appeared severely dehydrated. A water deprivation of 48 hours reduced their weight with 16 and 20 %. The plasma osmolality rose up to 346 and 368 mosm/kg H_2O respectively. The increase in plasma osmolality was 15 and 19 % and in plasma protein 6 and 17 %. The osmolality of urine did not reach higher than 736 and 837 mosm/kg H_2O (Table I). Plasma and urine urea concentrations were more than doubled, but urine urea excretion showed no increase before water became available by hydration. The changes in renal function are shown in Table II. Despite dehydration the urine flow rate decreased only to about half of the initial rate. When the reindeer were allowed to drink freely the urine flow rate rose strongly and, in spite of the still relatively

Acta physiol scand. 1977 100 340-346

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Responses of Reindeer to Water Loading, Water Restriction and ADH

By

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Received 25 January 1977

Abstract

VALTONEN M and L. ERIKSSON *Responses of reindeer to water loading water restriction and ADH* Acta physiol scand 1977 *100* 340-346.

Two female reindeer were hydrated by administration of (10 of b.w.t.) water into the rumen. The diuretic response was very fast and strong but the urea and electrolyte excretion were little affected. Dehydration was carried out by not giving the reindeer water for 48 h. This water deprivation caused a loss of up to [illegible] of their body weight. The urine osmolality did not exceed 840 mosm/kg H_2O, although the plasma osmolality rose from 300 to 346 and 368 mosm/kg H_2O respectively. The plasma and urine urea concentrations were elevated during dehydration, while the urine urea excretion did not increase. Urine sodium concentration did not increase. When the urine flow rate after two days of water deprivation decreased to half of the original the urine Na concentration instead of increasing, went down to half of the original. So did the potassium excretion. When ADH was injected intravenously into hydrated animals a dose of 30 mU of ADH was needed to induce antidiuresis or increased excretion of potassium. The reduced response to ADH and the low relative thickness of the medulla confirm the limited capacity of reindeer kidney to concentrate urine or to excrete a solute load. On the other hand, reindeer is able rapidly to excrete large amounts of water without affecting the electrolyte or nitrogen balance.

Key words. Reindeer hydration, dehydration, kidney function, ADH sensitivity

Water balance is primarily effected by controlling the ingestion of water and the excretion in the urine. When deprived of water the loss in urine is determined by the ability of the renal mechanism to concentrate urine The maximum urine concentration varies greatly among different species. Comparative studies of water and energy economy in ruminants have been carried out by Macfarlane *et al* (1971) They have observed that reindeer living in cold desert have the highest rates of water turnover In mammals there is a close relationship between the thickness of the inner zone of the kidney medulla and the renal concentrating ability (Schmidt Nielsen and O Dell 1961). In reindeer kidney the medulla is very (Sperber 1944) and urine osmolality rates measured in free living reindeer are also relatively small (Eriksson and Valtonen 1974) The maximum concentrating ability of the reindeer kidney has not been investigated. The diuretic response following oral administration of water and the response of kidneys to antidiuretic hormone (ADH) are other [illegible] nctions, appar[illegible]

II Effect of two days of water deprivation on renal function in the reindeer

	Urine flow rate ml/min		Renal excretion of						Osmolal clearance ml/min		Creatinine clearance ml/min	
			Urea µmol/min		Sodium µmol/min		Potassium µmol/min					
...er	M	H	M	H	M	H	M	H	M	H	M	H
...al	3.2	2.6	236	324	195	129	824	593	6.3	5.6	78	102
...deprived 24 h	2.3	1.3	321	166	164	44	518	426	4.9	3.0	76	72
...r deprived 48 h	1.8	0.9	295	218	54	20	430	255	3.8	2.1	54	56
...dration	5.6	10.1	460	511	178	81	262	373	4.7	5.1	91	106

...dration not changing, thus, the concentration in urine. It remained low after rehydration. ...ter two days of water deprivation the glomerular filtration rate as estimated from ...genous creatinine clearance, was reduced by 30 and 45% and osmolal clearance by ...nd 60 respectively. One day of water *ad libitum* almost restored the original values.

...DH sensitivity. The reindeer were hydrated in order to inhibit their endogenous ADH ...tion. Different amounts of ADH (5, 10, 20, 30, 40, 80 and 400 mU) were injected i.v. ...ated tests were made and the results of one of these expts. are presented in Fig. 2. ...y slight and brief reductions of water diuresis were obtained by 5 and 10 mU. Doses of ...r 30 mU of ADH were needed to induce negative C_{H_2O}.

...ome minor day to day variations in the response to a constant dose of ADH were observed. ...e basal level of C_{H_2O} was not always stable before the injections. Therefore the effect ...ADH for the dose response curve was expressed as the diminution of C_{H_2O} beneath

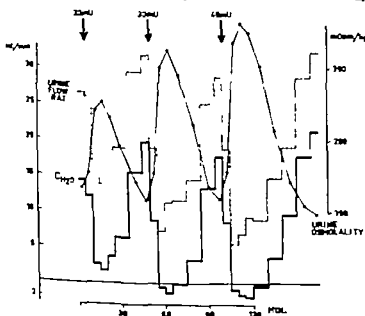


Fig. 2. Antidiuretic responses to graded doses of exogenous ADH in the reindeer M. Intravenous injections of ADH indicated by arrows.

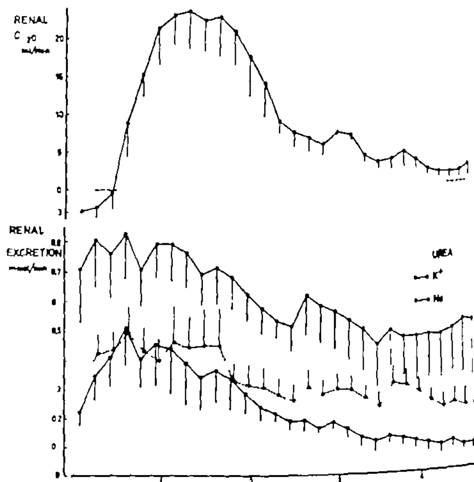


Fig. 1 Diuretic response f llowing administration of 6.5 l water given at the time 0 into the ru… reindeer The figure also shows the renal excretion of electrolytes and urea. Values given as mea… (n = 4)

high plasma osmolality water diuresis developed the next day The initial urine N… centration was 60 and 50 mmol/l and it went down to 30 and 20 mmol/l after 2 days… deprivation. After rehydration the concentration was 30 and 8 mmol/l respectiv… sequently the sodium excretion was greatly decreased but restored almost to the… after one day of water *ad libitum*. Potassium excretion was diminished to half of the…

TABLE I Changes in plasma od urine concentration after two days of water deprivation and re… in two reindeer M od H

	Plasma						Urine osmol… mosm…
	Osmolality mosm/kg H_2O		Total protein g/l		Urea mmol/l		
Reindeer	M	H	M	H	M	H	M
Normal	296	[illegible]98	74	70	6.7	4.7	585
Water deprived 24 h	3[illegible]5	334	74	75	8 9	5 0	703
W ter deprived 48 h	346	368	77	84	12.6	13.2	736
Rehydration	303	306	70	68	9.9	[illegible]	257

up to 20% of their b.wt. and plasma osmolality rose to 346 and 368 mosm/kg H_2O ... when deprived of water for 48 h. Dehydration to this extent must be considered ... However urine osmolality rose only about 800 mosm/kg H_2O. The maximum urine ... osmolality measured in cattle averaged 1300 (Weeth and Lesperance 1965), whereas in ... sheep and camel it is about 3 000 (cf. Macfarlane et al. 1956). According to our ... results, the reindeer is unable to excrete a high osmolal urine in comparison with other ... mammals. The limited concentrating ability of the reindeer must be closely related to the ... renal medulla as is the case in some other mammals (Schmidt Nielsen and O'Dell ... Sperber 1944).

In addition to concentrating urine in medulla, the kidney is able to reduce the loss of water ... by decreasing the glomerular filtration rate. In sheep this decrease was 50 ... after 5 days of water deprivation (Macfarlane et al. 1961) and in heifers 46 ... after 4 days (Weeth et al. ...). In reindeer the reduction was of about the same size. Because of the limited ability ... to increase urine osmolality the reindeer kidney like that of the bovine responded to water ... deprivation mainly by decreasing the glomerular filtration rate.

During water deprivation the kidney has to excrete salts and solutes despite the insufficient ... amount of water. Merino sheep for instance, a ruminant well adapted to dehydration can ... excrete urine sodium concentration higher than 500 mmol/l (Macfarlane et al. 1961). ... cattle, with poor concentrating ability can excrete urine with a sodium concentration ... higher than that of the plasma (Weeth and Lesperance 1965). The reindeer on the contrary ... did not excrete sufficient amounts of sodium when deprived of water and the concentration ... of sodium in urine did not increase. Instead, it went down to half of the original value. It ... is likely that the reindeer would have succumbed in this expt. if water restriction had been ... continued. Merino sheep can survive 5 days without water in hot climate (Macfarlane et al. 1961). Macfarlane assumed that the differences in survival depend on the ability to ... conserve water and to excrete sodium during dehydration.

Our observation that reindeer is very resistant to ADH agrees with the report of Macfarlane (1970/71). He has performed comparative studies of ADH sensitivity in other ruminants (Macfarlane et al. 1967). According to their results, cattle are less responsive to the ... antidiuretic action of ADH than sheep. Camel is the most sensitive, and responds even to ... ... ADH. However higher doses are needed for kaliuretic response. Renal sensitivity ... to ADH is proportional to the concentrating ability. Thus the high doses of ADH required ... for the reindeer to respond are related to its limited capacity to excrete high osmolar ... urine.

It appears from this study that the reindeer kidney has a very limited capacity to concentrate urine or to excrete a solute load. On the other hand, the ability of the reindeer kidney to excrete surplus water without losing solutes appears to be excellent. In fact this special ... feature is very convenient for freely roaming reindeer since their diet consisting of lush ... green vegetation in summer and mineral deficient lichen in winter may contain 75 to 90 % ... water. These reindeer may even appear somewhat overhydrated. However when extra rations ... are given the specific feature of the reindeer kidney must be taken into account.

This work was supported by ... grant from the Finnish Veterinary Medical Foundation.

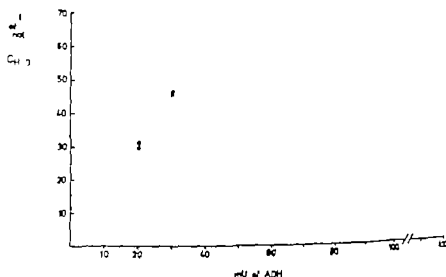


Fig. 3 Dose response curve to graded mounts of ex genous ADH in two reindeer The magnitude response is expressed as reduction f C_{H_2O} time (10 ml/ml ml)

+20 ml/min. The response was calculated by the reduction of C_{H_2O} being multiplied by time. Although there were some variations in the sensitivity between the two animals, slope of the curve was similar (Fig. 3) The doses which caused antidiuresis also cause slight increase in renal potassium excretion The relation of medullary and cortical leng of one reindeer kidney pair was measured and compared with a pair of sheep kid that of the reindeer being 0 86 and sheep 2.33

Discussion

The diuretic response following oral administration of water to reindeer was very fa compared with other ruminants investigated In reindeer diuresis began within 30 In cattle the average time between water loading and the onset of diuresis is 45 min a peak diuresis 150 min (Dalton 1964) In goat almost an hour is required to the onset of di after water loading (Andersson 1955) These divergent rates are presumably functions o water permeability of the rumen According to Macfarlane *et al* (1971), in cattle moves more rapidly from rumen to plasma than in sheep, and the process is very sl the camel Therefore, it is likely that the rapid onset of the water diuresis in reind partly due to water moving very rapidly from the rumen to blood

The urea and electrolyte excretion were little affected by water loading in reindeer W diuresis did not increase urinary excretion of the urea in cattle either (Weeth and Lespe 1965), but in sheep there was a distinct wash out of urea during diuresis (Schmidt Ni *et al* 1958) Reindeer seem to have a good ability to excrete surplus water without loss of electrolytes or nitrogen

During dehydration the plasma urea concentration increased in reindeer like in ruminants (Livingston *et al* 1962, Macfarlane *et al* 1961 Blanca *et al* 1965). The rel

p to 20 of their b.wt. and plasma osmolality rose to 346 and 368 mosm/kg H_2O ively hen deprived of water for 48 h. Dehydration to this extent must be considered However urine osmolality rose only about 800 mosm/kg H_2O The maximum urine lality measured in cattle averaged 1300 (Weeth and Lesperance 1965), whereas in n sheep and camel it is about 3 000 (*cf* Macfarlane *et al* 1956). According to our s, the reindeer is unable to excrete a high osmolal urine in comparison with other mals The limited concentrating ability of the reindeer must be closely related to the renal medulla as is the case in some other mammals (Schmidt Nielsen and O Dell Sperber 1944).

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Acta physiol. scand. 1977 100. 347–353

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Oxygen Consumption and Contractile Force during Vibrations of Cat Soleus Muscle

By

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Received 26 January 1977

Abstract

NG, B M. HALLBÄCK, R. SIVERTSSON and B. FOLKOW *Oxygen consumption and contrac tile force during vibrations of cat soleus muscle* Acta physiol scand. 1977 *100* 347 353

influence of longitudinal vibrations (50 Hz, 0 4 mm) on isometric twitch force development (4 Hz), l flow and oxygen consumption was studied in the acutely denervated soleus muscle of the anaesthetized t was found that the sinusoidal vibrations reduced the twitch amplitude by 60 per cent whereas oxygen mption and blood flow were lowered by 15 per cent only Similar reduction in twitch force was also ned by lowering the nerve stimulation intensity (4 Hz). This was associated with diminution in oxygen umption, the degree of hich was linearly related to the attenuation of active force, i the number of ated motor units The results are in agreement with previous observations as to the mechanical effect brations on active force in smooth and striated muscle They demonstrat that vibrations prevent the actile response with maintained high oxygen consumption hich adds further support to the hypothesis anded by Joyce *et al.* (1969) that vibrations cause increased rate of detachment of actin-myosin cross- . In addition appears possible that vibrations to some extent prevent formation of such cross-links.

gitudinal vibrations have been shown to reduce active force in various types of contrac tions such as skeletal muscle (Buchthal and Kaiser 1951 Matthews 1966, Joyce, Rack Westbury 1969), smooth muscle (Ljung and Sivertsson 1975, Ljung and Hallgren 1975) in myocardium (Vukas, Sivertsson and Ljung, in press). The mechanism which es the vibration induced inhibition of muscle force was suggested by Buchthal and er (1951) to be associated with the contractile mechanism. This hypothesis was further loped by Joyce *et al.* (1969) who proposed that actin-myosin cross-links are detached a increased rate when vibrations are applied. Indirect support for this tenet was recently ined in experiments with the anterior byssus retractor muscle of the Mytilus edulis re differentiated effects result when vibrations are applied during the "phasic" and ch phases, respectively (Ljung and Hallgren 1975) and in guinea pig taenia coli when lied during contraction or rigor respectively (Bose and Bose 1975).

f vibrations cause uncoupling of the contractile process, i.e. act at late stage in the cle cell, it would be anticipated that high energy demands prevail even when the external

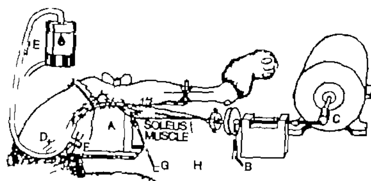


Fig. 1 Schematic illustration of experimental set-up

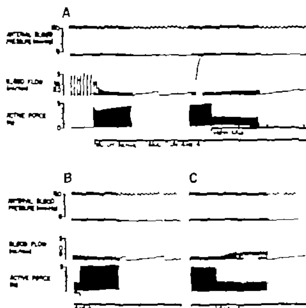
force output is reduced due to induced oscillations. In the present experiments blood flow and oxygen consumption were analyzed in the soleus muscle of the cat during recording of twitch force and the influence of vibrations upon these parameters was studied

Methods

The soleus muscle preparation used n these experiments has previously been described in detail (Folkow and H lcka 1968). Cats w th a body weight ranging between 3 0 and 4.0 kg were anesthetized with chloralose 50 mg/kg i.v after induction with ether The trachea was cannulated t permit free airway The skin of the left hind limb was dissected free and all its vascular supplies were ligated. The saphenous artery and vein were l gated and the thigh muscles were divided between ligatures just above the knee. The gastrocnemius muscle was carefully removed after ligation of all its arterial nd venous connections All branches of the popliteal artery and ein except those supplying the soleus muscle were l gated, so that the femoral artery and vein had ntact vascular connections with the soleus muscle only The distal part of the soleus muscle was dissected free from the peroneus muscles and the posterior part of the calcaneous bone at the insertion of the Achilles tendon was cut The motor fibres supplying the soleus muscle were sectioned and the peripheral ends placed n a small ring electrode f electrical stimulation. A hole was drilled through the tibia and the marrow cavity was plugged with grease and cotton to exclude venous drainage from the muscle via that route. The leg was then firmly anchored to an adjust ble anvil (A) Fig. 1) by a screw through the tibial h le. Throughout the dissection nd the experiment muscle temperature as monitored by means of superficial thermistor (H) and great caution was taken to keep muscle temperature constant and muscle surface moistened with Tyrode sol tion.

After the proximal part of the t bia had been immobilized the tendon of the soleus muscle was attached to a disk shaped force transducer (B) via a holder fixed by screws into the fragment of the calcaneus bone The isometric force transducer which was based on semico ductor transducer element (AE 803 AKERS Electronics, Norway) allowed to linear recording of force ncrements in the interval 0–50 N on a Grass polygraph. The force signal was also monit red on an oscilloscope for analysis of rates of vibration applied to the tendon duri g the experiment Vibrations were nduced by running the moto (C) at ariable speed and the rotary movement were transformed to longitudinal oscillations by means of a levered piston which was eccentrically attached to the motor axis. The vibration amplitude was determined by the distance between the piston lever and the center of the axis and was measured as the excursions of an illuminated spot on the transducer through dissection microscope with a micrometer eye-piece. The amplitude of the oscillations applied to the tendon amounted to ± 0.4 mm at the frequency of 50 H used in the experiments.

Arterial inflow pressure was monitored through cannula in the deep femoral rtery (D) After heparinization, the blood flow of the soleus muscle was measured by connecting the femoral vein t an optical drop recorder unit (E), and recorded on the polygraph. The venous outflow was returned to the animal via the right femoral ein. Venous blood samples for determination of xygen saturation were taken from a branch of the tubing (F). Arterial blood samples were drawn from the brachial artery O ygen saturation was determined spectrophotometrically n 0.8 ml hemolyzed blood, drawn during steady stat conditions. Hemoglobin concentration was determined at intervals during the experiment. Oxygen content of arterial



1. Recording of arterial blood pressure, venous outflow and isometric twitch force of the cat soleus muscle. A Muscle at rest, during activation at 4 Hz, supramaximal intensity and the effect of vibrations (50 Hz, 0 4 mm in amplitude. B. Cessation of ibrations Note rapid return of twitch amplitude. C Reduction of twitch force by submaximal (0 4 V) nerv stimulation intensity Not reduction in blood ... associated ith reduced umber of activated muscle units.

of venous blood as thus obtained and, since the blood flow through he muscle as recorded, oxygen consumption per minute could be calculated

The experimental protocol was as follows. Oxygen consumption, blood flow and contractile force ere measured during sequence of experimental situations at steady stat each as defined by stable blood flow and force of contraction The experimental sequence included 1) Muscle at rest,) Single twitches at 4 Hz, supramaximal stimulation intensity (4 V) 3) Single twitches as above and longitudinal ibrations (50 Hz, ±0 4 mm in amplitude) 4) Single twitches at 4 Hz, 4 V (same as 2) 5) Single twitches at 4 Hz but submaximal stimulation intensity (0 4 V), 6) Same as 7) 7) Muscle rest In each of 5 cats two complete sequences were obtained

Statistical analysis was performed by Student's t test for group comparison or pairing design Differences between mean values were considered significant for p 0 05

Results

Tracings of arterial blood pressure, venous outflow from the soleus muscle and contractile force recorded during one experimental sequence are illustrated i Fig. 2. The blood flow of the denervated muscular bed increased markedly from the resting level as single twitches were induced by nerve stimulation at 4 Hz with supramaximum intensity After twitch amplitude and blood flow had reached steady state levels, ibrations (50 Hz ±0 4 mm) were applied to the tendon while nerve stimulation with the same parameters was continued. In response to the length oscillations the twitch force was markedly reduced whereas blood flow remained almost unchanged It can be seen from the tracings t high paper speed that vibra-

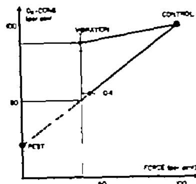
TABLE I Mean values ± S E. of soleus muscle blood flow oxygen consumption and force during different experimental conditions. The mean values are based on one observation in each animal during each of two experimental sequences (for details see "Methods").

Experimental condition	Blood flow ml/min 100 g	O_2 consumption ml/min 100 g	Twitch force (N)
Rest (n = 10)	12.6 ± 2.4	0.7 ± 0.1	0
Supramaximum stimulation intensity 4 V 4 Hz (n = 10) (control)	31 1 ± 2.9	3.0 ± 0.3	3 0 ± 0 4
Supramaximum stimulation intensity and vibrations 50 Hz (= 10)	27 6 ± 2.3	2.7 ± 0.3	1.1 ± 0 1
Submaximum stimulation 0 4 V 4 Hz (n = 10)	17 0 ± 2.3	1 6 ± 0 2	1.3 ± 0 1

tions not only reduced the amplitude but also decreased the duration of the individual twitches. Upon cessation of vibrations (Fig. 2 B) the contractile activity rapidly resumed the control pattern Next the nerve stimulation intensity was reduced from the supramaximum setting at 4 V to the submaximum value of 0 4 V (Fig. 2 C) Twitch force was lowered to approximately the same extent as by vibrations but in contrast to that situation, blood flow was now also significantly diminished Furthermore, the reduction in contractile force was not accompanied by any appreciable change in the duration of the twitch.

In Table I mean values for blood flow oxygen consumption and twitch force are summarized At an arterial blood pressure around 100 mmHg, which was largely constant throughout the experimental procedures, resting blood flow of the soleus muscle was 12.6 ± 2.4 ml/min 100 g tissue and oxygen consumption 0 7 ± 0 1 ml O_2/min 100 g. During motor nerve stimulation at supramaximum intensity the soleus muscle produced a twitch force of 3 N Blood flow then increased almost threefold and oxygen consumption approximately four times as an average of all recorded sessions. As the active muscle was then vibrated at 50 Hz the twitch force was markedly reduced whereas blood flow and oxygen consumption were only slightly affected When an equal reduction in force was instead induced by reducing the intensity of motor nerve stimulation, blood flow and oxygen consumption were significantly reduced both compared to supramaximum intensity stimulation and to the period when vibrations were allowed to reduce twitch force (see Table I). In each experimental sequence three periods of control stimulation were obtained (see "Methods"). The values obtained in these were entirely comparable and in Table I only the results of the first control stimulation are included Oxygen saturation of the venous blood was essentially unchanged during the experimental sequence

The values obtained for twitch force amplitude and oxygen consumption during vibrations or submaximum intensity stimulation, respectively were also expressed as a percentage of the corresponding values during the preceding control stimulation. As shown in Fig 3 about equal reductions in force were achieved by vibrating the soleus muscle when stimulated at supramaximum intensity and by reducing the intensity of motor nerve stimulation to 0 4 V In the latter case the metabolic requirement was reduced in proportion to the reduced force development since a linear relationship is obtained between oxygen consumption and force during high, low and zero activation of the soleus muscle. This is compatible with a



Oxygen consumption plotted against contractile force t and during twitches at 4 Hz. Values obtained during vibrations ("4 V ibration") or during submaximum lation ("0 4 V"), respectively have been expressed as ntage of the corresponding control stimulation at maximum intensity ("control, 4 V"). Thin lines drawn dicate extra amount of oxygen spent during vibrations given force development, mean ± S E., = 10.

ered number of activated motor units during submaximum stimulation. When vibra- s were applied to the active muscle, however oxygen consumption was 89 ± 5 per cent an SE, n 10) whereas twitch force only amounted to 38 ± 3 per cent of that during the ceding control period of supramaximum stimulation (Fig. 3). The small reduction in gen consumption was found to be statistically significant (p = 0 046) when tested by dent's t-test for pairing design. Blood flow was reduced to the same extent (89 ± 3 per t, p 0 003) during vibrations. It is clear that during vibrations the metabolic cost per tch of the soleus muscle is much higher than when twitches of comparable magnitude are uced by submaximum stimulation intensity

Discussion

e twitch force amplitude of the soleus muscle was reduced by more than 50 per cent when rations were applied but oxygen consumption and blood flow were only minimally uced The attenuation of contractile force by longitudinal oscillations of skeletal muscle s previously been reported as stated in the Introduction (Buchthal and Kaiser 1951 tthews 1966, Joyce *et al.* 1969). Qualitatively the same effects of vibrations can be in- ced in smooth muscle (Ljung and Sivertsson 1972, 1975, Ljung and Hallgren 1975, Bose d Bose 1975) and in cardiac muscle (Vukas *et al.*, in press). Since vibrations cause ibition in widely different types of muscle there are reasons to assume that all kinds of ively contracting tissues are sensitive to oscillating length changes. In previous studies on lated vascular smooth muscle (Ljung and Sivertsson 1975) and isolated papillary muscle ukas *et al.*, in press) it has been shown that the degree of inhibition depends on plitude as well as on frequency of vibration. Furthermore, it was shown in smooth uscle that the magnitude of the inhibitory effect, both in relative and absolute terms, pends on the prevailing levels of passive and active tension. That is to say that the active rce is inhibited to a greater relative extent in a muscle which is well prestretched and/or ghly activated than in one where these experimental conditions are not met (Ljung and vertsson 1975). In the present study no quantitative relationship was sought between varied ibration parameters and inhibitory response and it is not meaningful to make comparisons

between the sensitivity of the soleus muscle to vibrations and that of isolated vascular smooth muscle (Ljung and Sivertsson 1975) nor myocardium (Vukas et al. in press). In preliminary experiments a frequency of 50 Hz and an amplitude of ±0.4 mm peak-to-peak was found to cause at least 50 per cent reduction in twitch force of the soleus muscle. The vibrator was designed to deliver such vibrations but the actual oscillations of the tendon did contain higher frequencies as well

Blood flow and oxygen consumption of the denervated soleus muscle varied in parallel at steady state during the various experimental situations. This indicates that blood flow was adjusted by local mechanisms in response to metabolic demands. This conclusion is supported by our finding that venous oxygen saturation did not change markedly during the experimental sequence. Since vibrations inhibit isolated vascular smooth muscle it has been suggested that local vibrations in the case of turbulent blood flow or when working with vibrating tools may cause localized vasodilatation (Ljung and Sivertsson 1975 Sivertsson and Ljung 1976) In the present experiments it was not observed that vasodilatation occurred in the soleus muscle when its tendon was vibrated This by no means excludes the possibility that local dilatation can occur when vibrations are more directly applied to the vascular wall

The aim of the experiments was to elucidate whether vibrations cause an uncoupling effect in active muscle, i.e. if oxygen consumption at steady state would remain high while the mechanical response is reduced This was invariably found to be the case (Table 1, Fig. 3) The results demonstrate that the force output per unit oxygen consumed is markedly lowered by vibrations. The twitch force was also lowered by reducing the nerve stimulation impulse intensity A linear relationship was found between force and oxygen consumption when values obtained at rest submaximum and supramaximum intensity stimulation at 4 Hz were correlated (Fig 3) At rest the basic metabolic needs of the muscle correspond to approximately 25 per cent of the oxygen consumption at development of maximum twitch force at 4 Hz When 40 per cent of the tissue was activated by submaximum stimulus intensity twice as much oxygen was consumed as during rest This extra energy can theoretically be considered spent in the processes of activation internal work and tension development respectively since no external work is produced during isometric conditions The relative proportions are not known for the present experimental situation but by inference to analyses of heat production in skeletal muscle it can be assumed that approximately 30 per cent of the extra energy expenditure is connected with excitation and coupling mechanisms, and the remaining 70 per cent is used for internal work and tension development (for review see Mommaerts 1969 Abbott and Howarth 1973) When vibrations were applied to the soleus muscle the twitch amplitude was reduced by 60 per cent whereas the mean value for oxygen consumption was only lowered by 15 per cent (Table 1) It is evident that the muscle performs less efficiently when exposed to vibrations than when a corresponding tension development was obtained by activation of only 40 per cent of the muscle mass. Since it can be assumed that at most one third of the extra oxygen used during vibrations is spent as activation energy (see above) the main part of the difference between oxygen expenditure during vibrations and submaximum stimulation respectively would be due to interference with the efficiency of the contractile processes. This finding is entirely compatible

hypothesis that vibrations cause increased rate of detachment of actin–myosin (Goyer et al. 1969). In fact, it appears that it is only at such a late step in the n that vibrations could cause an uncoupling effect in view of the maintained high oxygen consumption. However the small reduction in oxygen consumption which l to be associated with vibrations of the active muscle may indicate that twitch x reduced only by increased rate of cross-link detachment but also to some extent tion of cross-link formation. Thus, the splitting of ATP associated with actin-teraction would be interfered with.

nk Lars Stage for construction of experimental equipment, Miss Ann Kjellstedt and M. Sten r able technical assistance and Mrs Kerstin Lundgren for secretarial aid. This work was supported dish Medical Research Council (No 14X-3444 and 14X-00016) and by AB Hässle.

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to derive from the sacral parasympathetic outflow (*nervi erigentes*). The neuro-
mission of erection is, however obscure (*vide* Klinge and Sjöstrand 1974 a). In our
repts on isolated field stimulated strips of the rp and the penile artery (pa) of bull
rived at the conclusion that acetylcholine (ACh) hardly is the transmitter which
ly relaxes the muscle cells. Thus, the inhibitory response to transmural nerve stimula-
was unaffected by botulinum toxin and hemicholinium (Klinge and Sjostrand 1974 a).
the present report we have extended our investigation to smooth muscle effectors of
ion in mammals other than the ox. Our aim was to obtain information on the basic
iology and pharmacology of these muscles. Special attention has been paid to their
itory innervation. Preliminary accounts of the present results have been presented
er (Klinge and Sjöstrand 1974 b, Sjöstrand and Klinge 1975).

Material and Methods

rial

material comprised rp from rat (adult Sprague-Dawley), dog, cat, horse, boar hog, elk (*A alces*), ram and goat, corpora cavernosa urethrae (ccu) from macaque (*Macaca irus s. fascicularis*, *philip-*pinensis), rabbit, guinea-pig, dog, cat and horse, and inflow resistance vessels (penile artery in the cavernous bodies of bull (*cf* Klinge and Sjostrand 1974 a). The rp of rat was defined as the ... the ventral part of the ischiococcygeus muscle (Gibson and Gillespie 1973). Although this muscle ... in the scrotum it was regarded as homologous to the rp of the other species because we ... few aberrant bundles of dog rp with similar scrotal insertion. Preparations from rat, cat and ... pig consisted of one side of the whole rp or ccu. Other preparations were strips of 5–20 mm length ... 1–3 mm width. The material was put in +4°C Tyrode or Ringer within 30 min from the death of the animals. With exception of the elk, the rp of which was obtained during the oestrus period in October, material from the other species was available throughout the year.

Data from elk are based on 9 strips obtained from 3 animals and those from goat on 8 strips from one ... buck. Data from other species are based on strips obtained from at least 5 animals. The equine material consisted of strips from 2 stallions and 5 geldings.

All strips were mounted in 35°C Tyrode aerated with 6.5 % CO_2 in O_2 and stretched (5–20 mN). The mechanical responses were recorded isometrically. For further details see Klinge and Sjostrand (1974).

Test of drugs

Organ baths of 5 ml were used. A rough characterization of the quality of the responses and the sensitivity of the preparations is given in Table I as follows. Contractile responses are indicated by +, relaxations by – and unresponsiveness by 0. The comparison of the effects is intended to be made in vertical rather than in horizontal direction. The threshold concentration for the most sensitive preparation to each compound is given. Each step in the grading reflects a difference in sensitivity of about 10 times. In preparations not contracting spontaneously the inhibitory effects were studied after contraction by compounds other than α-adrenergic agonists.

Field stimulation of nerves

Organ baths of 20 or 30 ml were used. Strips were mounted between two parallel platinum electrodes (5–20 mm long and 5–8 mm apart). Transmural stimulation of the nerves was performed with square wave pulses of 0.1–1.0 ms width and usually "supramaximal" voltage (15–35 V). "Supramaximal" voltage was defined as a voltage slightly larger than that evoking maximal ... tetrodotoxin (TTX, 3.1 · 10^{-6} M) ... responses. TTX-resistant contractile responses could easily be obtained in rp's of ... boar and ..., while such responses rarely could be provoked in the bull rp and most of the ccu's. Presumably these differences are due to differences in shunting of and resistance to current in nonnervous tissues rather than to differences in properties of the intramural nerves. Frequencies exceeding 5 Hz were not used unless frequency-response studies were performed. Usually the train length did not exceed 10 ...

All frequency–response analyses of the inhibitory response were performed on strips pretreated for 2

Acta physiol. scand 1977 100 354-367

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Comparative Study of Some Isolated Mammalian Smooth Muscle Effectors of Penile Erection

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Received 8 February 1977

Abstract

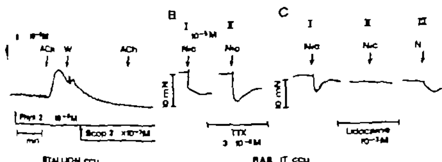
KLINGE, E. and N O SJÖSTRAND *Comparative study of some isolated mammalian smooth muscle effectors of penile erection* Acta physiol scand 1977 *100* 354-367

Spontaneous rhythmic activity responses to drugs and effects of field stimulation of nerves of the retract penis (rp) and/or corpus cavernosum urethrae (ccu) of macaque, rabbit, guinea-pig, rat, dog, cat, bear boar elk, bull ram and goat, as well as of the penile artery (pa) of bull were studied. A basic property of these muscles was automaticity Their responses to 5-hydroxytryptamine, histamine, adenosine phosphate prostaglandins E_1, E_2 and $F_{2\alpha}$, oxytocin, vasopressin, substance P bradykinin and angiotensin exhibited considerable species variations. Their excitatory innervation seems to be adrenergic. They also have an inhibitory innervation. In spite of comprehensive pharmacological analysis the inhibitory mediator remains obscure. The frequency-response relationship to inhibitory nerve stimulation was characterized by a rapidly achieved maximum at low frequencies, indicating high efficiency of the neuroeffector

Key words. Penis, smooth muscle, autonomic innervation

As first realized by Kölliker (1852), relaxation of smooth muscles, *i.e.* of those in the arteries supplying the cavernous bodies, of those in the walls of the cavernous spaces and of those in the retractor penis (rp) constitutes a most essential condition for initiation of penile erection. When these muscles relax the erectile tissue is engorged and the penis protrudes. When they contract the penis is relaxed and/or withdrawn Kölliker's concept has been experimentally proved by many investigators. Later studies also have shown that these smooth muscles receive an identical innervation (for ref see Klinge and Sjöstrand 1974). Therefore, there is reason to regard them as a *functional entity* *i.e.* smooth muscle effectors of penile erection All these effectors are not present in all mammals. Thus, the rp originating from the caudal vertebrae or the anal region and inserting at the distal part of the penis is lacking in *e.g.* primates, lagomorphs and many rodents. On the other hand, many artiodactyls are almost devoid of smooth muscle in their cavernous bodies.

The excitatory innervation of the smooth muscle effectors of erection derives from the lumbar sympathetic outflow and seems to be adrenergic. The inhibitory innervation seems



2. Cholinergic effects. *A* Scopolamine-susceptible contractile response to acetylcholine in ccu of ... physostigmine. *B*. Nicotine-induced relaxation of rabbit ccu is resistant to treatment with ... of tetrodotoxin (TTX) for 1 h. *C* Lidocaine blocks the relaxation, which slowly ... after washing. Nicotine was added at 1.5 h intervals. Tone (about 25 mN) was secured by ... (5 10⁻⁴ M).

... bovine muscles (Fig. 1 H). In some strips, especially of ram and goat, more complex ... with slow tonic waves and superimposed phasic contractions could be observed (... 1 C). The automaticity of the muscles was unaffected by TTX (3.1 10⁻⁴ M).

Only one out of 30 strips of the boar rp spontaneously showed signs of rhythmicity. The period between the contractions was 10 to 20 min. However strips of hog more frequently showed automaticity. This suggests that at least in this species automaticity of the rp is dependent on the hormonal state of the animal, as is the case with the smooth muscle of internal male accessories where castration promotes automaticity (Wilcke 1937 ... Porrelli and Valle 1969). In all muscles, even in the rp of boar NA (1 10⁻ M) often triggered automaticity.

The rp of cat and bull and the ccu of rabbit and cat usually developed high spontaneous tone ... some hours. Except for the rp of horse, boar and ram, all other preparations occasionally developed spontaneous tone (*cf* Ambache, Killick and Zar 1975).

Effects of known and putative neurotransmitters and other drugs (Table I, Fig. 1 and 2)

Table I summarizes the effects of the drugs. For explanation of the grading see Material and Methods.

Cholinergic effects. A Muscarinic About one third of the muscles were regularly contracted by ACh without signs of tachyphylaxis. The threshold concentration for the ... rp was about 5 10⁻⁴ M. Some canine rp:s were as sensitive but there was great variation between strips from different dogs. Preparations never contracted by ACh (5.5 10⁻⁴ M) in the presence of physostigmine (2.4 10⁻⁴ M) were considered insensitive. The contractile response was abolished by atropine or scopolamine (2.6 10⁻⁷ M Fig. 2 A). Thus, some of the muscles seem to be provided with excitatory muscarinic receptors. Muscarinic receptors probably located in adrenergic nerve terminals will be considered in a subsequent report (Klinge and Sjostrand 1977).

B. Nicotinic The effects summarized in Table I are all produced by the same concentration of nicotine, *i.e.* 4 10⁻⁴ M. All tissues except the rp of boar were relaxed. This

Fig. 1 Automaticity of isolated retractor penis (rp) or corpus cavernosum urethrae (ccu) ... mammals and of bull penile artery (pa) and the influence of drugs and nerve stimulation ... *A* Rhytmic activity in rat rp. *B* Continuous stimulation of inhibitory nerves (bar 0.1 ms) ... phasic rhythmic contractions in ram rp pretreated with bretylium (2.9 10^{-4} M) *C* Complex ... of automaticity in goat rp *D* Histamine relaxes goat rp without abolishing the automaticity *E* ... contraction induced by nerve stimulation in ccu of macaque which is spontaneously in tone. *F* ... slow rhythmicity in cat ccu. *G* Fast rhythmicity in ccu of stallion. *H* Low concentration of ... (IPN) abolishes rhythmicity in bull penile artery

to 4 h with guanethidine (1.3–3.4 × 10^{-4} M) or 6-hydroxydopamine (6-OHDA, 5.2 10^{-4} M) together ... ascorbic acid (3.1 10^{-4} M), *i.e.* half the concentrations used by Wadsworth (1973). In order to ... tone of the preparations noradrenaline (NA, 1–6 × 10^{-6} M) was sometimes added.

We also investigated the ccu of rat, boar, bull and ram but they were probably devoid of smooth ... because they did not respond even to 3 10^{-4} M adrenaline or to field stimulation. The rigid c... cavernosa penis of rabbit, cat and dog were also omitted because they showed so tiny responses ...

Drug

Acetylcholine chloride (Roche), physostigmine salicylate (Sandoz), atropine sulphate (ACO), ... bromide (Sigma), nicotine bitartrat (BDH), hexamethonium bromide (May & Baker), mecamyl... hydrochloride (MSD), 1-adrenaline bitartrate (Sigma), 1-noradrenaline bitartrate (Sigma), dop... hydrochloride (Sigma), isoprenaline sulphate (Abbot), phenoxybenzamine hydrochloride (Smith, K... Fr) chlorpromazine chloride (Medica), thioridazine chloride (Sandoz), propranolol hydroch... (ICI), guanethidine sulphate (Ciba), bretylium tosylat (Wellcome), 6-hydroxydopamine hydroch... (Fluka), ascorbic acid (Merck), 5-hydroxytryptamine creatinine sulphate (Fluka), methysergide ... (Sandoz), lysergic acid diethylamide d tartrate (LSD Sandoz), histamine hydrochloride (Sigma), ... benzamine (Recip), mepyramine maleate (Pharma Rhodia), cyproheptadine hydrochloride (M... metiamide (Smith, Kline & Fr), cimetidine (Smith, Kline & Fr), adenosine triphosphate (Se... dipyridamole hydrochloride (Boehringer), substance P (synthetic, Beckman), bradykinin (BRS ... Sandoz evaporated and redissolved in saline) isoleucine[5]-angiotensin (Beckman), valine[5]-angiot... (Ciba), oxytocin, vasopressin and lysine-vasopressin substances (Ferring), prostaglandins E ... $F_{2\alpha}$ (Astra), indomethacin (MSD), lidocaine chloride (Astra), tetrodotoxin (Sankyo), lithium ch... (Merck), g-strophantin (ouabain, Sandoz), colchicine (Star), SKF 525 A (proadifen, Smith, Kl... Fr), and metoclopramide (Lundbeck). We thank the companies indicated by an asterisk for gen... supply of the drugs.

Results and Comments

Automaticity and spontaneous development of tone (Fig. 1 and 3 G)

Except in the boar rp, rhythmic contractions could be observed in all of the preparatio... The spontaneous contractions occurred at intervals of about 1–3 min. The character... the contractions varied from rapid phasic in the ram (Fig. 1 B) to more semitonic in ...

PGE_1	$PGF_{2\alpha}$	Subst. P	Brady-kinin	Angio-tensin	Vaso-pressin	Oxy-tocin
●		0	0	(+)		0
		+	+	0	+	0
	+	●	0	(+)	0	0
		(+)	+	0	+	0
	0	+++	+++	+++	0	0
			+++			
		++	+++	0	0	0
( )	●	++	+++	(+)	0	0
●	●	++	+++	0	+	0
	+	0	0	−	+	0
	++	+	+	( )	0	0
		0	+	−	++	+
( )	+	0	+	++	+++	+
	++	0	0	+	+	0
	++	+	++	(+)	++	(+)
++	++	+	●	−	++	+

tio of about 1 3 10. The threshold concentration for A was 1–8 10^{-8} M The rp:s are slightly more sensitive to α-adrenergic stimulation than the ccu:s except the equine ones in which the reverse relationship existed. The contractile effects were abolished by phentolamine (3–30 10^{-7} M) or phenoxybenzamine (3–30 10^{-8} M).

β Beta-adrenergic. Preparations contracted spontaneously or by compounds other than adrenergic agonists were relaxed by isoprenaline (IPN). The threshold concentration was 10 10^{-10} M for the penile artery of bull (Fig. 1 H) and for the rp of cat, boar and ram depending on whether the preparations were spontaneously active or contracted by an agonist. Occasionally the ccu of guinea-pig was contracted by large doses of IPN (cf. Klinge 1972) and this effect was reversed by phenoxybenzamine. Phenoxybenzamine also potentiated the effects of A and NA. Propranolol (3 4 10^{-8} M) blocked the A NA and IPN-induced relaxations.

5-Hydroxytryptamine (5HT). Several of the muscles exhibited both excitatory and inhibitory responses to 5HT. They were tachyphylactic and therefore not systematically analysed. In the feline tissues the contractile response was blocked by methysergide (1 10^{-8} M) or LSD (1 10 10^{-8} M) but the relaxing effect was not blocked by these compounds nor was it blocked by lidocaine (7 4 10^{-4} M) or TTX (1 6 10^{-6} M). The threshold concentration of 5HT for the cat rp was about 2.5 10^{-8} M.

TABLE I Responses to various agents of the isolated retractor penis muscle and the corpus cavern (ccu) of some species as well a of the bovine penile artery

	Cholinergic effects		Adrenergic effects		5HT	Hi
	Muscarinic	Nicotinic	NA (α)	IPN (β)		
Retractor penis						
Rat	+ +	–	+ + +	–	+	
Dog	+	–	+ + +	– –	+	+
Cat	(+)	–	+ + +	– – –	+ –	
Horse	+	–	+ + +	– –	(+)	+++
Boar	0	+	+ + +	– – –	–	+
Elk	(+)		+ + +			
B ll	+	–	+ + +	– –	+ +	++
Ram	0	–	+ + +	– – –	(+)	+
Goat	0	–	+ + +	–	+	
C						
Macaque	(+)	–	+ + +	– –	–	+
Rabbit	0	–	+ + +	– –	+	++
Guinea-pig	0	–	+ + +	(–)	+ +	++
Dog	(+)	–	+ + +	– –	(–)	+
Cat	(+)	–	+ + +	–	+ –	++
Horse	+	–	+ + +	– –	+	+++
Penile artery						
Bull	+	–	+ + +	– – –	+	++

Symbols. + = contraction, – = relaxation, + – = contraction followed by relaxation. Bracket ind responses inconstantly obtained with large doses. 0 = no effect. NA = noradrenaline, IPN isoprena 5-hydroxytryptamine, HI = histamine. For threshold concentrations and further explanations see the from bovine tissues are taken f om Klinge and Sjöstrand (1974 a).

phenomenon was even without pretreatment demonstrable in the rabbit ccu, bull rp the feline muscles, while most other tissues required pretreatment with *e.g.* 6-OHDA. the boar rp nicotine produced contraction only whereas relaxation could be produced the rp of hog. The relaxation was always characterized by a rapid onset occasionally pre ceded by a brisk contraction The maximum depth of the relaxation did not exceed th of the relaxation produced by field stimulation (see below). The relaxation show considerable tachyphylaxis and at least 30 min had to elapse before it could be repeate It was blocked by hexamethonium (2.8 10⁻⁴ M) or mecamylamine (2.5 10⁻⁴ M). It w also blocked by lidocaine (1.8–11 10⁻⁴ M Fig. 2 C) In fact the effect of nicotine w more susceptible to lidocaine than the effect of field stimulation. The relaxation was n however blocked by TTX (6 3 10⁻⁶ M Fig. 2 B *cf* Toda 1976). The sensitivity lidocaine of the nicotinic effect suggests a neuronal rather than a muscular point of attac Its resistance to TTX suggests that nicotine does not activate "fast sodium channel (Narahashi 1975). The effect of several other drugs on the nicotine-induced relaxatio has been analyzed earlier (Klinge and Sjöstrand 1974 a)

Adrenergic effects A Alpha-adrenergic. All tissues were vigorously dose-dependent and without tachyphylaxis contracted by adrenaline (A), NA and dopamine in a do

PGE2	PGF2α	Subst. P	Brady-kinin	Angio-tensin	Vaso-pressin	Oxy-tocin
0	+	0	0	( )		0
		+	+	0	+	0
		0	0	(+)	0	0
		(+)	+	0	+	0
	0	+++	+++	+	0	0
			+++			
		++	+++	0	0	0
( )	0	++	++	( )	0	0
0	0	++	+++	0	+	0
	+	0	0		+	0
	+	+	+	( )	0	0
	+	0	+	+−	++	+
( )	+	0	+	++	+++	+
	++	0	0	+	+	0
	++	+	++	( )	++	(+)
+	++	+	0	+	++	+

tio of about 1 3 10. The threshold concentration for A was 1–8 10⁻⁸ M. The rp:s were slightly more sensitive to -adrenergic stimulation than the ccu:s except the equine ones in which the reverse relationship existed. The contractile effects were abolished by phentolamine (3–30 10⁻ M) or phenoxybenzamine (3–30 10⁻⁸ M).

β Beta-adrenergic. Preparations contracted spontaneously or by compounds other than β-adrenergic agonists were relaxed by isoprenaline (IPN). The threshold concentration was –10 10⁻¹⁰ M for the penile artery of bull (Fig. 1 H) and for the rp of cat, boar and ram depending on whether the preparations were spontaneously active or contracted by an agonist. Occasionally the ccu of guinea-pig was contracted by large doses of IPN (cf. Klinge 1972) and this effect was reversed by phenoxybenzamine. Phenoxybenzamine also reversed the effects of A and NA. Propranolol (3.4 10⁻⁸ M) blocked the A NA and IPN-induced relaxations.

5-Hydroxytryptamine (5HT). Several of the muscles exhibited both excitatory and inhibitory responses to 5HT. They were tachyphylactic and therefore not systematically analyzed. In the feline tissues the contractile response was blocked by methysergide (1.1 10⁻⁸ M) or LSD (1 10 10⁻⁸ M) but the relaxing effect was not blocked by these compounds nor was it blocked by lidocaine (7.4 10⁻⁴ M) or TTX (1.6 10⁻⁶ M). The threshold concentration of 5HT for the cat rp was about 2.5 10⁻⁸ M.

Histamine The threshold for the equine tissues and the cat rp was about 1 10⁻⁴ All effects of histamine were blocked by phenbenzamine (8 10⁻⁴ M). In the ... cimetidine (4×10^{-4} M) opposed neither the contractile nor the relaxing actions of ... Thus, it seems that all observed effects were due to stimulation of H_1-receptors.

Adenosine triphosphate (ATP) Except in the cat rp the effects of ATP were ... and tachyphylactic. The threshold concentration for the cat rp was about 2 10⁻⁴ The inhibitory effect on the ccu of macaque was uninfluenced by dipyridamole (1 10⁻ M), lidocaine (7.4×10^{-4} M) and TTX (3.1×10 M) But in the bull rp excitatory effect was slightly augmented by dipyridamole (1 10⁻⁴ M). Tissues responding to 2×10^{-4} M ATP were considered insensitive.

Prostaglandins E E_1 and $F_{2\alpha}$. In all tissues the actions of PGE and PGE_1 were ... The threshold concentrations for the feline muscles were about 2 10⁻⁴ M Tissues responding to 3 10⁻⁴ M were considered insensitive.

The threshold concentration for the long lasting excitatory effect of $PGF_{2\alpha}$ was ab 5 10⁻⁴ M in the cat rp and most of the ccu:s. Tissues not responding to 1 10⁻⁴ M ... considered insensitive

The effects of the prostaglandins were characterized by slow onset, long duration variable degree of tachyphylaxis.

Substance P The threshold concentration for the boar rp was about 4 10⁻¹⁰ M. effects were dose-dependent without tachyphylaxis. Tissues not responding to $4 \times 10^{-}$ were considered insensitive

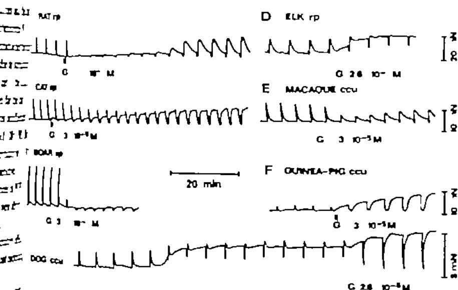
Bradykinin The threshold concentration for the rp:s of the artiodactyls was ab $1 \times 10^{-}$ M the porcine and the ovine rp being slightly more sensitive than the ot... The contractile effect was dose-dependent without tachyphylaxis. Muscles not respon... to 2 10 M were considered insensitive.

Angiotensin The threshold concentration for the rp of boar and hog was about 5 1 M for both isoleucine⁵ and valine⁵-angiotensin The porcine rp:s and the ccu:s of guinea and dog responded in a dose-dependent way without tachyphylaxis. Tachyphylaxis observed in the less sensitive muscles. Preparations not responding to 5 10⁻ M ... considered insensitive.

Vasopressin and oxytocin The threshold concentration of arginine-vasopressin for dog ccu was about 1 10⁻⁴ IU/ml. The vasopressin-induced contractions showed ... onset and were long lasting. Tissues not responding to 1×10 IU/ml were consid... insensitive. Neither lysine- nor arginine-vasopressin contracted the porcine rp:s tissues which were very sensitive to vasopressin were also slightly contracted by oxyt... (1×10^{-4} IU/ml) The effects of the posterior pituitary hormones exhibited consider... tachyphylaxis

Effects of field stimulation of nerves (Fig. 1 B 1 E and 3–8)

The common response to field stimulation of a preparation which was in moderate sp... taneous tone was triphasic, *i e* 1) an initial contraction (= excitatory response) followed 2) a relaxation (= inhibitory response) and then by 3) a secondary contraction. In prep... tions being in low tone often only the first contraction was seen or the response appea...



3 Blockade of the excitatory and unmasking of the inhibitory response by guanethidine (−G.). Immediate suppression of the excitatory response in the rat rp. Note the gradual development of the tory contraction (2 Hz, 2 ms, 10 s). *B* Slowly developing effect in cat rp (0.5 Hz, 0.1 ms, 4 s). rp (4 Hz, 0.5 ms, 4) pretreated with 6-OHDA (5.2 10^{-4} M). *D* Triphasic response with secondary contraction before guanethidine in rp of elk (4 Hz, 0.5 ms, 10). *E* In ccu of prominent secondary contraction develops after guanethidine (2 Hz, 2 ms, 10). *F* Immediate in ccu of guinea-pig (2 Hz, 0.5 ms, 10 s). *G* Spontaneous rise in tone in canine ccu unmasks bitory response and secondary contraction. Guanethidine produces further rise in tone and ing of inhibitory response (5 Hz, 2 ms, 10 s).

a biphasic contraction. In preparations being in high tone often only the inhibitory ponse was seen (*cf* Klinge and Sjöstrand 1974 a)

Excitatory response A Pharmacology The response was blocked by guanethidine 4 10^{-4} M, Fig. 3), bretylium (1 3 10^{-4} M *cf* Luduena and Grigas 1966) or 6-OHDA 2 10^{-4} M). The rp of boar usually required combined treatment with 6-OHDA and guanethidine (Fig. 3 C), while combined treatment was not needed in the rp of hog. As a rule the blockade by guanethidine developed more slowly in the rps than in the ccus probably this as a matter of penetration. Guanethidine increased the tone of those preparations ich had the tendency to develop spontaneous tone. 6-OHDA contracted all the preparations within 30 min and full blockade of the excitatory response developed within 2-4 h. These results confirm the adrenergic nature of the excitatory innervation of the muscles (*cf* Klinge and Sjöstrand 1974 a).

In this context it should be mentioned that we noted no certain guanethidine-like effect of LSD (1 100 10^{-6} M) on the excitatory response in the bull rp. But LSD contracted most preparations probably by a direct muscle effect and thereby uncovered the inhibitory response (*cf* Gillespie and McGrath 1975).

B Frequency-response relationship Sensitive preparations responded to single shocks (Fig. 4) Full confluence of individual responses usually occurred at 0.1–1 Hz. It occurred

Histamine The threshold for the equine tissues and the cat rp was about 1 10 1
All effects of histamine were blocked by phenbenzamine (8 10^{-4} M). In the feline tw
cimetidine (4 10^{-4} M) opposed neither the contractile nor the relaxing actions of histam
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Effects of field stimulation of nerves (Fig. 1 B, 1 E and 3–8)

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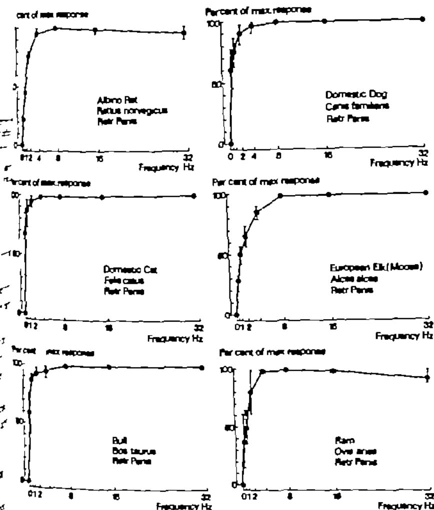


Fig. 5. Frequency–response relationship of the isometric inhibitory response evoked by "supramaximal" field stimulation of the rp. Length of train 10 s, interval between stimulations 5–6 min. The excitatory response is suppressed by guanethidine or 6-OHDA (see the text). Each curve illustrates means and range of three preparations from different animals. Note that in all species maximum inhibition is obtained with 8 Hz. The fading of the inhibitory response seen at higher frequencies in some preparations is presumably mainly due to breakthrough of the excitatory response.

stimulation period or the voltage was decreased the curves were shifted to the right (*cf* Klinge and Sjöstrand 1974 a). A more detailed analysis revealed some differences between the rp's and the ccu's. Thus, when a fixed number of pulses (5–25) was delivered at different rates the rp:s usually responded with the same degree of inhibition to frequencies between 0.5 and 10 Hz, while in the ccu:s the inhibition was more frequency-dependent (Fig. 7 and 8). This tendency was especially clear in the rabbit ccu where the responses were very frequency-dependent up to 4–6 Hz, and less clear in the ccu of macaque and guinea-pig,

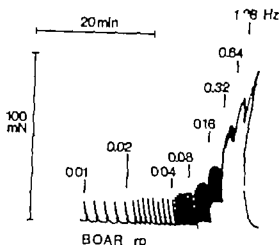


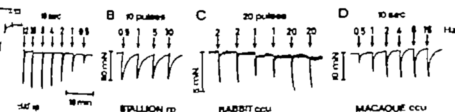
Fig. 4 Excitatory responses to single shocks [illegible] summation and confluence of responses [illegible] increasing frequencies. Summation starts at [illegible] and confluence starts between 0.64 and 1.3 [illegible] Stimulation was interrupted [illegible] reached upper level of recording. Note [illegible] tractions.

at lower frequencies in preparations with semitonic rhythmic contractions than in th with rapid phasic automaticity (see above) Because the excitatory response was cut do by the succeeding inhibitory response, which could not be selectively blocked (see belo) meaningful further frequency–response analysis of the excitatory response could no performed (see Klinge and Sjöstrand 1974 a)

Inhibitory response A Pharmacology Like the excitatory response the inhibitory repor was abolished by TTX (3 1–31 10^{-6} M) and lidocaine (5–15 10^{-4} M). This sugges neural origin. The response was not blocked by the following drugs (the highest cont trations tested are given) atropine (1 4 10^{-4} M) scopolamine (2.6 10^{-4} M), hex methonium (1 4 × 10^{-4} M), mecamylamine (4.9 10^{-5} M), d tubocurarine (1 4 10^{-4} M) metoclopramide (3 10^{-4} M), phenoxybenzamine (2.9 10^{-4} M) phentolamine (3 × 10^{-4} M) methysergide (2.1 × 10^{-4} M) LSD (1 10^{-4} M) cyproheptadine (6.2 10^{-4} M), mepyr mine (2.5 10^{-4} M) phenbenzamine (7 9 10^{-4} M), metiamide (4 1 10^{-4} M), cimetid (4 0 10^{-4} M) chlorpromazine (2.5 10^{-4} M), thioridazine (2.5 10^{-4} M), indometha (5 6 10^{-6} M), lithium (2.4 × 10^{-4} M) ouabain (1 10^{-4} M), SKF 525 A (5 6 10 [illegible] for 12 h) or colchicine (5 10^{-4} M for 12 h) The response was not influenced by dipyrid mole (1 9 10^{-4} M) Thus, our pharmacological results gave no hint of the nature of th inhibitory mediator

B Frequency–response relationship Since the excitatory response could be blocked b adrenergic neuron blocking agents a more detailed analysis of the inhibitory respons was possible. All preparations responded to single shocks. Except in the pa of bull th frequency–response curves to 10 s stimulation were indeed very steep, with maximu inhibition at 2–8 Hz (Fig. 5 6 and 7) The curve of the cat rp was steepest and that of th elk rp the least steep In Fig. 5 and 6 the results from boar goat and stallion are omitted the first mentioned because a breakthrough of the excitatory response at 4 Hz and higher rates could not be prevented, and the latter two because of too small material. However in all the strips studied from stallion gelding, hog and goat maximum inhibition occurred at 4–8 Hz.

If the length of the trains was increased the curves were shifted to the left. If the



1 Recordings of the inhibitory responses to field stimulation. Methodology as in Fig. 5. *A*. Rat rp maximum inhibition at 8 Hz but fading at 32 Hz. *B* Stallion rp. When constant number of pulses is applied the inhibition shows little or no variation. *C*. Rabbit ccu. Note the frequency-dependence constant number of pulses (1 20) is applied at varying frequencies. *D* Ccu of macaque. Note the ...ity with the record in *A*.

...ulation and the tone of the muscle. It is likely that it is a true rebound phenomenon (Klinge and Sjöstrand 1974 a).

Discussion and Conclusions

...a comparative study the presence of similarities between organs and species is a most ...ial matter because it allows more generalized conclusions. Fundamentally all the ...ooth muscle effectors of penile erection exhibit characteristics of single unit smooth ...cle in having automaticity. Their excitatory innervation is apparently adrenergic, ...ey respond to single excitatory impulses. They are very sensitive to exogenous NA ...hough they have a fairly dense adrenergic innervation (*cf* Klinge and Sjöstrand 1974 a, 77). Automaticity combined with dense excitatory innervation and high sensitivity to ...excitatory transmitter must constitute advantages for muscles usually kept in tone, *i.e.* ...en the penis is relaxed.

The boar but not the hog differed from the other species with respect to automaticity ...the rp. Perhaps this is related to the unusually long (about 30 min) copulation time ...this species. At least a low degree of automaticity could facilitate long lasting ...traction of the muscle.

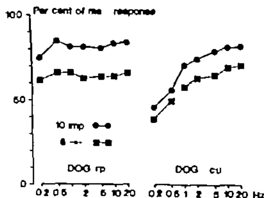


Fig. 1 Comparison of frequency-dependence of the inhibitory responses in the rp and ccu ...one and the same dog. Both preparations ...ken after guanethidine (1.3 × 10⁻⁶ M). ...ote frequency-independence in the rp at ...requencies above 0.5 Hz and frequency-dependence in the ccu at frequencies below 1 Hz. Frequency plotted on logarithmic scale.

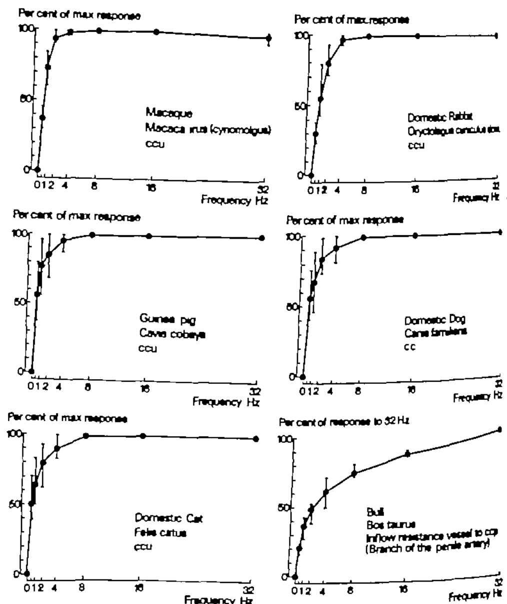


Fig. 6. Frequency–response relationship of the isometric inhibitory response evoked by "supramaximal" field stimulation of the ccu. For comparison, data from inflow resistance vessel to the corpus cavernosum penis (ccp) of bull is included. Methodology as in F g. 5 As in the rp (Fig. 5), maximum inhibition achieved also in the ccu within 8 Hz but not in the branch of the penile rtery

which usually responded with the same degree of inhibition to frequencies above 2 H In the bull pa maximum inhibition was not obtained with 10 s stimulation even at 32 H (Fig. 6 Klinge and Sjöstrand 1974 a) Finally it should be stressed that maximum inhibitio produced by field stimulation never implied maximum feasible relaxation of preparation that were in high tone. In rhythmically contracting preparations the inhibitory respons implied suppression of automaticity (Fig. 1 B)

Secondary contraction A secondary contraction (Fig. 1 E, 3 A, D and E) was not see in all preparations. When occurring it showed strong dependence on the degree of inhibitor

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…, E. and N. O. Sjöstrand, Suppression of the excitatory adrenergic neurotransmission … possible … of cholinergic nerves in the retractor penis muscle. *Acta physiol. scand.* 1977 100 368–376.

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The muscles showed variability in the presence of receptors for and in response to different biological agents such as 5HT histamine ATP some prostaglandins and polypeptides (Table I). The present results do not indicate any generalized role of these compounds in regulating the tone of the muscles. Presumably most smooth muscles of mammals possess receptors for various agonists which are of similar significance for the muscles as are vestigial organs for the animals, i.e. the receptors are present for developmental, evolutionary and genetic reasons rather than for functional purposes.

All of these smooth muscles seem to have an inhibitory innervation. The neurotransmitter remains, however, unknown. It is hardly ACh. The muscarinic action on the muscles, if present, is contractile, not relaxant. The nicotinic relaxations are likely to be due to activation of inhibitory nerves rather than to a direct action on the muscle cells. Furthermore, the inhibitory response to field stimulation is resistant to nicotinic and muscarinic blockers as well as to hemicholinium and botulinum toxin (cf. Klinge and Sjöstrand 1974a).

Although the inhibitory mediator remains obscure, certain information about the inhibitory response to field stimulation has been obtained. The steep frequency-response relationship as well as the rapidly achieved and limited maximum seems to be a general property of the rp:s. Possibly one explanation of this phenomenon is a high degree of occupancy of muscular receptors by the mediator occurring rapidly as a result of nerve stimulation. In any case our findings indicate that the rp with its inhibitory nerves is a highly efficient neuroeffector unit. Our data suggest that the ccu is a somewhat less efficient neuroeffector unit and the bull pa still less efficient. These differences might be related to differences in intimacy between nerves and receptors in effector cells. In the ... differences in efficiency of the units might be compensated by geometrical factors. The ... the functional response of inflow resistance vessels (pa) to the cavernous bodies is related to the fourth power of the muscle length and the tissue mass of the media and intima, ... of the muscles in the cavernous bodies (ccu) to the second or third power of the muscle length while the functional response of the rp is related to the first power of the muscle length only.

We wish to thank Mrs Annika Roséen for excellent assistance in making the figures and Mrs Kerstin Westling for excellent technical assistance. The courtesy of the personnels at the Department of Experimental Surgery Karolinska Hospital Stockholm, and at the city slaughter houses of Stockholm and Helsinki is gratefully acknowledged. The study was supported by grants from the Magnus Bergvall Memorial Fund and the Swedish Medical Research Council project number B 76-04X-04750 (to N. ...) and by a scholarship from the Medical Research Council of the Academy of Finland (to E. K.)

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rmore, electronmicroscopy of the bull rp has revealed axons with small agranular tic vesicles of the type generally ascribed to cholinergic nerves (Eränkö, Klinge and rand 1976).

the basis of the effects of exogenous ACh on the isolated field stimulated bull rp we suggested that cholinergic nerves in this muscle may act by muscarinic suppression of citatory adrenergic neurotransmission and thereby promote erection (Klinge and rand 1974). The present investigation was initiated by the observation on a strip of rp of elk that physostigmine suppressed the excitatory adrenergic response to field lation; atropine restored the response. Since field stimulation is likely to stimulate all s of nerves and physostigmine inhibits destruction of ACh released from nerves, this rvation supported our suggestion. Further investigations on rp:s from more convenient mals showed that the canine rp was the most suitable preparation for the study of the t of cholinesterase (ChE) inhibitors on adrenergic neurotransmission.

is report presents evidence indicating that the function of cholinergic nerves in the rp as well as in the rp of some other species may in fact be suppression of the excitatory ergic neurotransmission. Preliminary accounts of the present study have been nted earlier (Klinge and Sjöstrand 1976, Sjöstrand and Klinge 1977).

Material and Methods

rial

ctor penis (rp) muscles of dog, rat, cat, horse (stallion and gelding), swine (boar and hog), elk, bull, and goat were used. For further details concerning the material see Klinge and Sjöstrand (1974, 1977).

rements of acetylcholine (ACh) and noradrenaline (NA)

ay of ACh was performed on the superfused frog rectus abdominis muscle. Extraction and deter ion was done as described by Klinge (1970 b). NA was determined with the trihydroxyindole method ding to Chang (1964). Extraction, absorption and elution were performed as described by Sjöstrand Swedin (1970). Except on the canine rp all estimations were done on pieces weighing about 0.5 g cut the middle part of the muscle. When the ACh content of the dog rp was estimated the muscle was into two halves, i.e. anterior (preputial) and posterior (anal). When the NA content of the dog rp analysed the muscle was divided into three parts, i.e. anterior, middle and posterior. The most caudal which contains striated fibres was always omitted. In Table 1 the average concentrations of the two owards the whole dog rp are presented.

hanical responses to transmural nerve stimulation and drugs

procedure and the conditions were the same as in the preceding report (Klinge and Sjöstrand 1977). In the frequency–response studies were performed the procedure was as follow. Strips of the dog rp allowed to accommodate for 2 h. During this period the preparations were stimulated with 2 Hz for at 5 min intervals. Frequency–response analysis was then performed with 10 s stimulations at 5 min vals starting with 0.5 Hz and ending with 16 Hz. Thereafter physostigmine (2.4 $\times$ 10^{-7} M) was added. preparation was stimulated with 2 Hz for 10 s at 5 min intervals during 1 h before the frequency–response analysis was repeated. Scopolamine (2.6 $\times$ 10^{-7} M) was then added and the frequency–response analysis was repeated after 15 min. Control expts. were performed under identical conditions.

The influence of ACh, physostigmine and scopolamine on the contractile response to exogenous NA of canine rp was investigated in a 5 ml bath as follows. A dose of NA (1×10^{-7} to 1×10^{-6} M) giving stable responses was chosen. The NA was left in contact with the tissue for 90 s at 5 min intervals. ACh was added and the other drugs 5 min before NA.

Acta physiol. scand. 1977 100. 368-367

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Suppression of the Excitatory Adrenergic Neurotransmission; a Possible Role of Cholinergic Nerves in the Retractor Penis Muscle

By

ERIK KLINGE and NILS O SJÖSTRAND

Received 8 February 1977

Abstract

KLINGE, E. and N O SJÖSTRAND *Suppression of the excitatory adrenergic neurotransmission a possible role of cholinergic nerves in the retractor penis muscle* Acta phys scand 1977 *100* 368–376

The excitatory adrenergic response to transm ral nerve stimulation of the isolated retractor penis (r dog, rat, cat, horse, boa elk, bull, ram nd goat, as well as the evoked release of ³H from dog rp loaded with H-noradrenaline were studied I untreated p eparations low concentration of scopolam could markedly increase the excitatory drenergic response. On the contrary physostigmine suppressed this response and so did acetylcholine. Scopolamine or atropine totally prevented these pressions. They also immediately restored the suppressed responses in spite of co tinuous presence physostigmine and/ acetylcholine. In the rat rp there was no suppression of the response by ph stigmine. Physostigmine decreased the evoked release of H and this effect was counteracted scopolamine. The rp of dog, gelding, boar hog, bull and ram contained considerable amounts of adrenaline and also of acetylcholine It is a gested that one action of cholinergic nerves in the muscarinic suppression of the excitatory adrenergic neurotransmission.

Key words. Penis, smooth muscle, adrenergic nerves, chol nergic nerves

The retractor penis (rp) is one of the smooth muscle effectors of penile erection. I present in many subprimate mammals When the muscle relaxes the penis protrudes. Wh it contracts the penis is withdrawn under the skin All data support the concept that excitatory innervation is adrenergic. But the mediator of its inhibitory innervation unknown. It is unlikely that acetylcholine (ACh) is the mediator which relaxes the mus cells by a direct action upon them (*vide* Klinge and Sjöstrand 1974 1977).

However there is evidence indicating that the muscle receives postganglionic cholinerg nerve fibres Thus, the bull rp contains significant amounts of ACh and exhibits acet cholinesterase (AChE) activity (Klinge 1970 b) Strongly AChE positive nerves are pres in the rp of bull (Klinge, Pohto and Solatunturi 1970) and dog (Bell and McLean 197

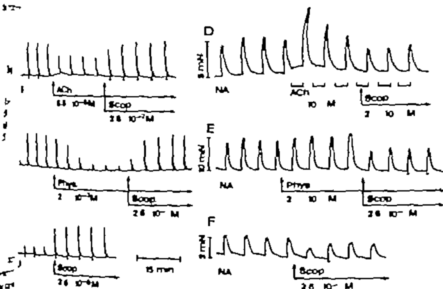


Fig. 1 Dog rp. Effects of ACh, physostigmine and scopolamine on the excitatory adrenergic response to transmural nerve stimulation (A, B and C) and on the effect of constant dose of exogenous noradrenaline (D, E and F). A Nerve stimulation (NS, 2 Hz, 1 ms, 10 s, at dots). The response is suppressed by ACh. ... B, Physostigmine inhibits the response to nerve stimulation (2 Hz, 10). It is ... by scopolamine. C A low concentration of scopolamine markedly enhances the excitatory response to nerve stimulation (3 Hz, 10 s) in an untreated preparation. D The response to noradrenaline (1.0×10^{-6} M, brief bars) is enhanced by ACh (bars). E Physostigmine enhances the response to ... F Scopolamine by itself reduces the response to NA (1.1×10^{-7} M) which is also seen in ... E.

... in the anterior middle and posterior parts of the canine rp, respectively. This is in accordance with the finding of Bell and McLean (1970) that there were no regional differences in the distribution of adrenergic nerves.

The NA concentration was twice as high in the rp of castrated hog compared with that of the boar. Presumably this is explained by a reduction of nonneuronal tissue as is the case in internal male accessories of castrates (*cf* Sjöstrand and Swedin 1976). The NA and ... concentrations of the bull rp agree with earlier results (Klinge 1970 a, b).

Effect of ACh, ChE inhibitors and antimuscarinic agents on the excitatory adrenergic response of dog rp to field stimulation (Fig. 1 and 2)

The adrenergic nature of the contractile response of the rp to transmural nerve stimulation has been established (Klinge and Sjöstrand 1974, 1977). ACh ($1 \times 10^{-} - 10^{-4}$ M) contracted the rp and suppressed the excitatory response (Fig. 1 A) whereas the effect of exogenous NA was enhanced (Fig. 1 D). Scopolamine ($1 \times 10^{-} - 10^{-4}$ M) counteracted these effects of ACh.

Physostigmine ($1 \times 10^{-7} - 10^{-4}$ M) invariably suppressed the excitatory response to nerve stimulation (Fig. 1 B) while it enhanced the effect of exogenous NA (Fig. 1 E). Scopolamine (... 10^{-4} M) immediately restored the contractile response to nerve stimulation

TABLE I Acetylcholine and noradrenaline concentrations in the retractor penis muscle of some dom mammals (mean ±S.E.).

	Dog	Horse (Gelding)	Swine		Bull	Ram
			Boar	Hog		
ACh μg/g	19 4±8 7 (4)	0.30±0 09 (4)	0.50±0.08 (7)	0 66±0 03 (5)	0 98+0.12 (7)	[illegible] (7)
NA μg/g	[illegible]1±0 3 (5)	1 1 ±0.2 (4)	3[illegible] ±0 7 (6)	6 8 ±0.9 (5)	3 5 ±0 7 (5)	6 1 (7)
Approximate average molar ratio ACh NA	11 1	1 3	11	1 9	1 3	1.3

Number of test animals in parentheses.

Evoked release of H from dog retractor penis preloaded with H NA

Strips of about 1 cm length taken from various parts of the muscle were incubated at 35°C for 1 Tyrode solution containing H NA (1-norepinephrine-7 H NEN), corresponding to a radioactive 2.5 μC/ml, and ascorbic acid (1 10⁻⁴ M). After repeated washings the strip was mounted in a perspex bath with platinum electrodes (10 mm long and 6 mm apart) in the wall. Tyrode solution with 6.5% CO_2 in O_2 and kept at 35°C was passed continuously from below and sucked off from surface at rate of 2 ml/min. In addition to the ascorbic acid the solution contained desipramine (5 10 M) and normetanephrine (1 10⁻⁴ M) in order to prevent uptake of released NA (cf. 1971). The effluent was collected in 2 ml fractions, the radioactivity of which was measured by counting 1 ml aliquots in Packard scintillation spectrometer using 10 ml Instagel® (Packard) as counting medium. The radioactivity in the whole tissue at the end of the experiment was determined after homogenization in 15 ml of 0 4 M perchloric acid. The rise in efflux of ³H evoked by field stimulation was calculated against the calculated total ³H present in the tissue at the beginning of the stimulation (Farnebo Hamberger 1971 Stjärne 1973) In control stimulations the peak fractional increase exceeded background by at least 70

Nerve stimulation was performed at about 15 min intervals with 100 rectangular shocks (1 ms, 2 Hz) Before the experiments were started the preparation was allowed to equilibrate for 1 h, during which three stimulations were performed.

Statistical analysis of the data was performed with standard formulae. Significance of difference ³H excretion was tested by Student t-test. A p value of 0 05 or less was considered significant.

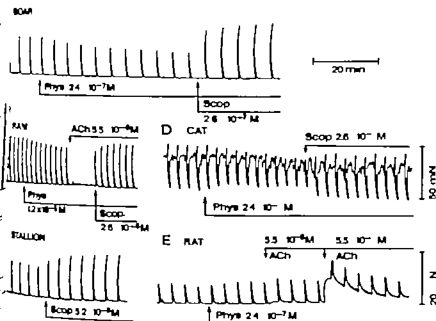
Drugs

Physostigmine salicylate (Sandoz), neostigmine bromide (Leo), pyridostigmine bromide (Roche), acetylcholine chloride (Roche), scopolamine bromide (Sigma), atropine sulphate (ACO), bretylium bromide (May & Baker), mecamylamine hydrochloride (MSD), 1-noradrenaline bitartrate (Sigma), desipramine hydrochloride (Ciba-Geigy), dl-normetanephrine hydrochloride (Sigma) and ascorbic acid (Merck).

Results and Comments

Acetylcholine and noradrenaline concentrations (Table I)

The rp:s contained high concentrations of NA. The ACh concentrations were also high and in the dog rp impressive. The posterior part of the dog rp contained ACh 3[illegible] 14.2 μg/g and the anterior part 3 6±1 1 μg/g. This regional distribution of ACh differs from that of AChE-positive nerves which, according to Bell and McLean (1970), are more frequent in the anterior part. The NA concentrations were 2.4±0.5 2.4±0 4 and 1 6



3 Effect of ACh, physostigmine and scopolamine on the excitatory adrenergic response to field stimulation of the isolated rp of some species other than the dog. *A* Boar. Stimulation. 2 Hz, 0.5 ms, ... 5 min intervals. Physostigmine suppresses the response. Scopolamine not only restores but ... enhances it (*cf.* Fig. 1 B). *B*. Ram. Stimulation. 1 Hz, 0.1 ms, 3 at 2 min intervals. Physostigmine ... the response. It is abolished by ACh and restored by scopolamine. Note the absence of any ... effect of ACh. *C*. Stallion. Stimulation. 2 Hz, 0.5 ms, 10 at 4 min intervals. The response is ... by low concentration of scopolamine (*cf.* Fig. 1 C). *D*. Cat. The muscle is spontaneously in ... Stimulation: 1 Hz, 2 ms, 4 at 3 min intervals. Physostigmine reduces the excitatory as well as ... inhibitory response. Scopolamine restores both responses. *E*. Rat. Stimulation: 2 Hz, 2 ms, 10 at ... intervals. Physostigmine and low concentration of ACh do not suppress the response. A higher ... of ACh considerably contracts the muscle. In spite of this the excitatory response is ... relaxed.

Scopolamine in low concentration (1.5 10⁻⁸ M) produced an enhancement of the excitatory response to nerve stimulation in some preparations of horse (Fig. 3 C) and boar ... direct relaxant action of scopolamine and atropine was less prominent than in the ... rp. But in all preparations the direct relaxant effect of atropine was considerably ... pronounced than that of scopolamine.

... the bull rp physostigmine usually produced a slight enhancement of the excitatory response to field stimulation. However this preparation was usually contracted by physostigmine (Klinge and Sjöstrand 1974).

In the cat rp physostigmine suppressed not only the excitatory but also the inhibitory response to field stimulation. Also this latter effect was counteracted by scopolamine (Fig. 3 D). Suppression by physostigmine of the inhibitory response was not observed in the rp of any other species.

In the rat rp ... obtained no evidence for muscarinic suppression by physostigmine of the excitatory adrenergic response to field stimulation (Fig. 3 E).

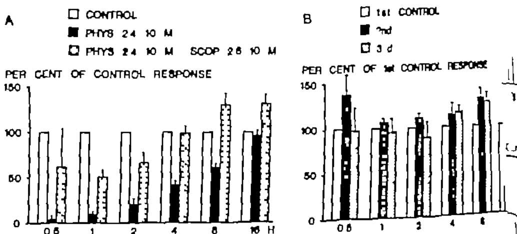


Fig. 2. Dog rp *A* The frequency dependence of the suppression by physostigmine of the excitatory re... to field stimulation Note that the response to 0.5 Hz is almost abolished. *B*. Corresponding ... experiment. In both figures mean ±S E., n=4

(Fig. 1 B) The effect of physostigmine was dependent on the frequency and numb... stimuli *i.e* it was greater (Fig. 2 A) and also more rapid in its onset at low freque... However by increasing the concentration of physostigmine also the responses to ... frequencies could be markedly suppressed. Similar effects were obtained with pyridostig... (3 8 10⁻ M) and neostigmine (3 3 × 10 M)

In certain preparations low concentrations (1–5 × 10^{-4} M) of scopolamine conside... enhanced the contractile response to nerve stimulation (Fig. 1 C) Scopolamine had a d... depressant action on the dog rp, manifested as a reduced response to exogenous ... (Fig. 1 D–F) and other nonmuscarinic contractile agents. It also relaxed muscles ... were in spontaneous tonic contraction Like scopolamine atropine (1 10^{-4} 10^{-4} ... counteracted the effect of physostigmine on nerve stimulation. Atropine, however ... a far more pronounced depressant action on the smooth muscle

The effects of physostigmine and scopolamine on the excitatory response to ... stimulation were unaffected by pretreatment with hexamethonium (2.8 10^{-4} – 1 4 10^{-4} ... or mecamylamine (4.9 10^{-4} – 2.5 10^{-4} M)

Effect of ACh, physostigmine and antimuscarinic agents on the excitatory adrenergic response of rat cat horse boar elk bull ram and goat rp to field stimulation (Fig. 3) With exception of the rat rp, ACh (1 10 – 10^{-4} M) had no or a weak direct contrac... effect on the preparations (Klinge and Sjöstrand 1977) In all preparations ACh suppres... the contractile response to low frequency stimulation. This effect could be drastic, espec... in preparations pretreated with physostigmine (1 10⁻ – 10^{-4} M) as *e.g* in Fig. ... Scopolamine (1 × 10^{-4} – 10^{-4} M) or atropine (1 10^{-4} – 10^{-4} M) immediately restored ... excitatory response to nerve stimulation

Physostigmine (1 × 10⁻ – 10^{-4} M) reduced the contractile response to field stimula... in the rp of cat, horse, elk, ram, goat and some bulls. This effect was, however rarel... marked as in the dog rp Scopolamine or atropine restored the contractile response (... 3 A and D).

s of scopolamine and physostigmine on the evoked release of ^{3}H from dog rp loaded with ^{3}H-NA are in favour of this concept.

The existence of inhibitory muscarinic receptors in adrenergic nerve terminals in various organs of different species is well documented by previous workers (Lindmar, Loffelholz and Muscholl 1968, for further references see *e.g.* Muscholl 1973, 1974 and Vanhoutte 1976). The higher effectiveness at low frequencies of the muscarinic suppression of the release of the adrenergic transmitter has been reported (*e.g.* Kirpekar, Prat and Wakade 1975). As autonomic nerves are supposed to operate *in vivo* at low frequencies, this supports a possible functional significance of the muscarinic effect, as also does the fact that cholinergic and adrenergic axons often run tightly intertwined in peripheral tissues (*e.g.* Jacobowitz, Falck and Sporrong 1970) including the bull rp (Eränkö *et al.* 1976).

Since in the rp ACh and NA do not seem to work as pure physiological antagonists on the smooth muscle effector cells the present results may also be taken as a support of the more generalized principle that one type of autonomic fibre may inhibit the transmitter release from another type (see *e.g.* Stjärne 1975).

The rp of the laboratory rat differed from the rps of the other species and showed no sign of muscarinic inhibition by physostigmine of the adrenergic neurotransmission. In this context it should be mentioned that Gillespie (1972) found no AChE activity in the rat anococcygeus muscle from which the rp takes its origin in this species. This points to notable species differences in autonomic innervation and function, as also does the potentiation by physostigmine of the inhibitory response to field stimulation found in the cat rp only. Finally it should be stressed that field stimulation implies a simultaneous activation of all types of nerves at the same rate. This is hardly the case *in vivo* and limits the interpretation of the physiological significance of the results.

We wish to thank Mrs Annikki Rosén for excellent assistance in making the figures and Mrs Kerstin ... for excellent technical assistance. The courtesy of the personnels at the Department of Experimental Surgery, Karolinska Hospital, Stockholm, and the city slaughter houses of Stockholm and ... is gratefully acknowledged. The study was supported by grants from the Magnus Bergvall Foundation Fund and the Swedish Medical Research Council project number B 76-04X-04753 (to N. S.) and by a scholarship from the Medical Research Council of the Academy of Finland (to F. K.).

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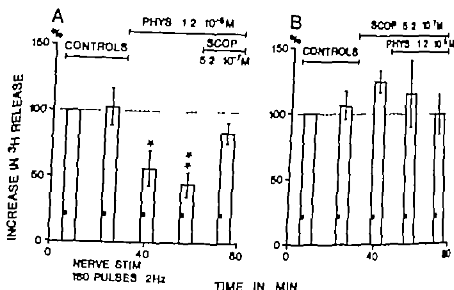


Fig. 4 Effect of physostigmine and scopolamine on the ^3H release evoked by field stimulation of ... preloaded with ^3H NA *A* Physostigmine significantly reduces the ^3H release and scopolamine ... (n = 5) *B* Scopolamine prevents the effect of physostigmine (n = 3). The ^3H release is expressed in p... of the first control (mean ± S E.) * = $p < 0.05$, ** = $p < 0.01$ (differences from second control).

Effects of physostigmine and scopolamine on ^3H relase evoked by field stimulation from dog rp preloaded with ^3H NA (Fig. 4)

After 20 min exposure to physostigmine the ^3H release due to field stimulation decreased by more than 50%. After addition of scopolamine the fractional release o... was raised towards the control level (Fig. 4 A) Pretreatment with scopolamine preve... the effect of physostigmine (Fig. 4 B). In one experiment scopolamine by itself incre... the evoked release of ^3H by more than 50%.

Discussion

The rather high ACh concentrations found in the rp of various species further support introductorily mentioned evidence for a cholinergic innervation of this muscle. The aim ... the present study was to define a possible functional role of the cholinergic nerves. W... respect to the effects of exogenous ACh on the rp two tentative roles for the choline... nerves may be outlined. 1) They might constitute a component of the excitatory innervat... or 2) contribute to the relaxation by suppression of the adrenergic neurotransmiss... (Klinge and Sjöstrand 1974) The first alternative seems unlikely because the contrac... response is weak, inconstant or absent (Klinge and Sjöstrand 1977) Furthermore, ... cholinergic nerves were a part of the excitatory innervation one should regularly obt... an augmented excitatory response to field stimulation after ChE blockade. The seco... alternative on the contrary is supported by the present results, particularly the findir... that a low concentration of scopolamine can enhance the adrenergic response to fi... stimulation of nerves and that this response is suppressed by physostigmine Also ...

Acta physiol. scand. 1977 100. 377–381

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Electrical Activity of the Rat Uterus during Early Pregnancy and Abortion

By

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Received 22 February 1977

Abstract

...LO, A. and A. E. KÄRKI. *Electrical activity of the rat uterus during early pregnancy and abortion.* Acta physiol. scand. 1977 *100* 377–381

...rical activity of the rat uterus was recorded for 1 h daily on days –9 of pregnancy with 6 electrodes ...ronically implanted in the left uterine horn. In 10 untreated and 7 ergocornine treated rats, activity ...reased frequency and intensity during the first days of pregnancy. After day 3 activity was present in ...about 30 % of electrodes as compared to 85 % on day 2. Abortion was induced by ergocornine injected ...day 5. In these rats the frequency of bursts increased on days 8 and 9. During the peak of activity the ...ajority of bursts spread in the cervical direction.

Key words: Uterus electromyography, pregnancy, abortion, chronic recording, ergocornine

...the fourth day of pregnancy in the rat, ova enter the uterus for implantation which ...rs on day five (Psychoyos 1973). During the preimplantation period the uterine motility ...s down in the rabbit (Fuchs 1972) and weak, slowly spreading bands of contractions can ...seen in the uterus (Boving 1971). They are assumed to be responsible for the spacing of ...blastocysts. The general decline of the uterine contractile activity has been recorded by ...intrauterine balloon (Fuchs 1972). Intraluminal balloons passing through the uterine ...d may however interfere with the uterine function at the time of implantation, when the ...rus is particularly sensitive to mechanical irritation. Furthermore the balloon method ...y allows analysis of activity in one or a few points while regional differences may play an ...ortant role in spacing blastocysts and guarding against premature expulsion of blasto-...s. Analysis of initiation and spreading of electrical activity in the rat uterine horn is ...ible with chronically implanted electrodes (Talo and Kärki 1976). We have used that ...thod to study changes of uterine activity during early pregnancy and induced abortion. ...gocornine administered on the fifth day of pregnancy blocks prolactin release which is ...essary to support the corpus luteum and thus results in a decrease of progesterone level ...peripheral plasma leading to abortion in the rat (Morishige and Rothchild 1974).

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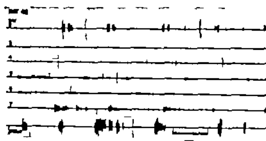


Fig. 2. Decline, disappearance and reappearance of electrical activity during early pregnancy and induced abortion. Activity recorded in the middle of the uterine horn with [illegible] electrode. Note that activity was also recorded on day 5 prior to ergocornine treatment.

[illegible]rsts of low amplitude and short duration appeared in one or several electrodes in the middle of the uterus.

The decline of electrical activity was best seen in the frequency of bursts (Fig. 4) and in the number of rats in which activity was recorded. The decrease of frequency of electrical activity took place from day 3 to day 4 but remained unchanged after that in the untreated group. In the ergocornine treated group the frequency increased on day 7. In all rats electrical activity was recorded in some or all electrodes in the middle of the uterine horn (electrodes [illegible]) on days 2 and 3. In four out of 12 rats activity was recorded from some of these electrodes on days 4 and 5 and in three on days 6, 7 and 8. Also the number of electrodes in which electrical activity was recorded dropped significantly from 86.2% to 23.7% from day 3 to day 4 ($p < 0.001$, t-test) but remained unchanged after that. Fig. 2 illustrates an example of [illegible] where electrical activity was recorded on day 5 prior to ergocornine treatment. While such an increase was found in a few rats, it was not statistically significant. The amplitude of bursts decreased from day 2 onwards (Fig. 5). Variations between individual rats were large, therefore the values are presented as means of individuals rather than group means. The duration of the bursts also declined from day 2 to day 3 in those few rats where any activity was recorded and was variable after that (Fig. 6).

Site of initiation, direction and distance of spread of electrical activity were analysed on days 4 and 5 and compared with the foetal locations. No consistent relationship was detected.

In ergocornine treated rats a higher percentage of bursts spread in the cervical rather than the ovarian direction at the time of highest activity on days 7, 8, or 9. On average the percentage of bursts spreading in the cervical direction was lowest at the ovarian end of the horn (6[illegible]) and highest at the cervical end (85%). Both uterine horns were empty in ergocornine treated rats. In the untreated rats significantly less foetuses were found in the left than in the right horn, 2.5 ± 1.93 (mean, SD) and 6.0 ± 2.6 respectively (p [illegible] 0.01). In three

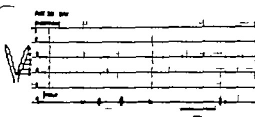


Fig. 3. Recording of electrical activity on day 9 [illegible] electrodes implanted in the same horn. A schematic diagram on the left illustrates positions of the electrodes with respect to the fetuses on day 9.

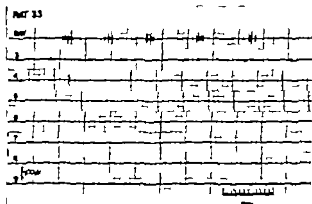


Fig. 1 Decline and disappearance of ele[ctrical] activity in the rat uterus during early [preg]nancy. Recording on 8 consecutive days with the same electrode in the middle of uterine horn.

Material and Methods

Each morning vaginal smears of Sparague Dawley rats were taken and examined for the presence of sperms. When positive, the rats were aseptically operated upon the same afternoon and this day counted as day 1 which corresponds with the day of oestrus during the cycle. 17 rats were anaesthetized by ... Nembutal (30 mg/kg) injected i traperitoneally Additional ether anaesthesia was used when ... During midventral laparotomy 6 teflon coated stainless steel wire (SSJT Med ... Corp. N.Y. ... meter 0.003" coated diameter 0.0045") electrodes, insulated at the tip, were implanted in the myometr[ium] of the left uterine horn as described earlier (Talo and Kä ki 1976). Electrodes were passed through myometrical wall using a 26 gauge hypodermic needle as a trocar A small area of the electrodes, ... free of insulation was placed inside the myometrium and the electrodes were secured in position by ... their tips back The whole procedure was done under a preparation microscope. The electrodes ... implanted at even distances, the first bei g located t the ovarian end 3 mm from the utero-tubal junct[ion] and the sixth at the level f bifurcation of the uterine horns. Ground and reference electrodes were ... to the area of incision or to the left mammary gland The electrodes were passed through the abdom[inal] wall, led under the skin to the neck and ttached to 8-pin plug. The last 5–8 cm of the electrodes ... passed through a polyethylene catheter which together with the electrodes, was rigidly glued to the ... Either the plug or the catheter was sutured t the skin at the neck. 1 94 out of 10 implanted electr[odes] activity was recorded during some day of recording.

After operation the rats were caged singly nd kept in their own cages during recording from 10 ... a m. A flexible cable connected the electrodes to inputs of a Grass 7P (inkwriting, curvilinear polygr[aph]). Low frequency f ltering (time constant 0 04 or 0.015 s) was used to eliminate drifts of the baseline. The ... recording was made on the day following operation, day 2. Recordings were made da ly until day 8 and autopsy performed on day 9 At a topsy the number of foetuses and the location of the electr[odes] with respect to them were determined.

Ergocornine hydrogen maleinat (Sandoz) was dissolved in 70 % ethanol on the day of use. Six of th[e] rats were given a single subcutaneous inject n of 1 mg n 0.5 ml at 3 p m. of day 5 of pregnancy (lab... as day 6 by Morishige and Rothch ld 1974). I jected at this t me nd quant ty t effectively results in a[bor]tion (Morishige and R thchild 1974). At the time of utopsy all the treated rats had aborted

Results

A gradual decline of electrical activity during early pregnancy is shown in Fig. 1 A sim[ilar] decline and a subsequent increase following ergocornine treatment is shown in Fig. 2. F[rom] day 3 onwards electrical activity appearing in the middle of the uterine horn did not sp[read] through the whole uterine horn. Sometimes activity of higher amplitude, beginning at e[ither] end, spread through the whole horn even when the activity was otherwise low Such ac[tivi]ties appeared in periods of bursts. Fig. 3 illustrates one period of bursts of higher ampli[tude] in the cervical end when the bursts did not spread through the uterine horn. Simultaneo[usly]



Daily variations of the mean duration of bursts of electrical activity in individual rats in the middle ... the mean horn

...rated primarily in the cervical direction. This suggested that abortion was a result of ... coordinated activity as during abortion at late pregnancy.

Ergocornine treatment on day 5 pregnancy results in a sharp decrease of serum progesterone level in 4 h and abortion in 48–72 h (Morishige and Rothchild 1974). The increase in frequency of spike bursts took place at about 48 h after injection suggesting a ...ship between progesterone level and evolution of activity. Also during abortion induced by prostaglandin or by ovariectomy in late pregnancy decline of progesterone level and ...ization of activity preceded abortion (dePalva and Csapo 1972).

Casteels and Kuriyama (1965) found that the resting membrane potential of the rat uterine smooth muscle increased during pregnancy and reached the highest level on the 15th ... day. It appears that the increase of resting membrane potential is too small during the first few days of pregnancy to explain the drastic decline of activity thus suggesting that other regulatory factors are involved.

In over 90 % of implanted electrodes activity was recorded on some day of recording. Although it was possible to determine the overall frequency accurate measurement of amplitude and duration was not always possible due to EKG interference. Implantation of electrodes in pairs certainly would have reduced interference but it was undesirable due to the small size of the rat uterus. Even 6 electrodes may have had some harmful effects on the uterus as suggested by a smaller number and size of foetal swellings in the horn bearing electrodes than in the control horn.

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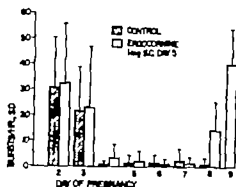


Fig. 4 Decline of frequency of bursts of electrical act during early pregnancy and an increase following th facient injection of ergocornine. Histograms indicate values, vertical bars S.D

rats the left uterine horn was empty and in a few others the size of swelling of the horn at foetal area was smaller than that on the right side.

Discussion

Our results showed that the electrical activity decreased in frequency and intensity dur early pregnancy In the rabbit uterine activity recorded by an intraluminal balloon increas after coitus and started to decrease after 48 h (Fuchs 1972) The electrical activity of the during early pregnancy was similar to that recorded in a normal oestrus cycle (Talo & Kärki 1976) Activity on day 2 corresponded to that of metoestrus and days 3 and 4 to th of dioestrus although no long bursts of activity were recorded No significant increase activity characteristic of the proestrus phase, was found on day 5 in pregnant rats.

We were unable to establish any definite correlation between initiation or spread electrical activity on day 5 and location of foetuses. It appeared that a recording lasting 1 h was too short to establish such a correlation. Furthermore activity was present only a few rats. This may result from the fact that low frequency filtering was used It has bee shown recently by Osa and Katase (1975) that the circular muscle layer of the rat uter during the middle of pregnancy displays slow waves of depolarization and not spike burs as does the longitudinal muscle layer However in late pregnancy the circular muscle laye activity also consists of spike bursts. If the circular muscle layer of the uterus, which is mo likely to be involved in the transport of blastocysts, displayed similar slow waves durin early pregnancy these would not have been recorded Therefore our results do not refute th relationship between electrical activity and spacing of blastocysts. It is interesting that th spread of electrical activity during the peak of activity following ergocornine treatmen

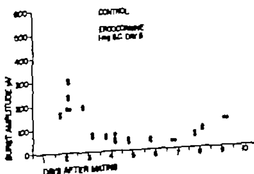
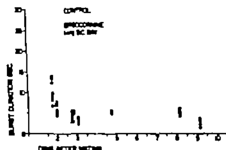


Fig. 5 Daily variations of mea amplitude of bursts of electrical activity in individual rats I the middle of the uterine horn.



...ily variations of the mean duration of bursts ...ed activity in individual rats in the middle ...rine horn.

...ed primarily in the cervical direction. This suggested that abortion was a result of ...ed coordinated activity as during abortion at late pregnancy

...nutrient treatment on day 5 pregnancy results in a sharp decrease of serum pro... ...ne level in 24 h and abortion in 48–72 h (Morishige and Rothchild 1974). The in... ...e in frequency of spike bursts took place at about 48 h after injection suggesting a ...between progesterone level and evolution of activity. Also during abortion induced ...or by ovariectomy in late pregnancy decline of progesterone level and ...ion of activity preceded abortion (dePalva and Csapo 1972).

...a and Kuriyama (1965) found that the resting membrane potential of the rat ...smooth muscle increased during pregnancy and reached the highest level on the 15th ...th day. It appears that the increase of resting membrane potential is too small during ...first few days of pregnancy to explain the drastic decline of activity thus suggesting that ...regulatory factors are involved.

...over 90% of implanted electrodes activity was recorded on some day of recording. ...ugh it was possible to determine the overall frequency accurate measurement of ...tude and duration was not always possible due to EKG interference. Implantation ...ctrodes in pairs certainly would have reduced interference but it was undesirable due ...small size of the rat uterus. Even 6 electrodes may have had some harmful effects on ...ns as suggested by smaller number and size of foetal swellings in the horn bearing ...trodes than in the control horn.

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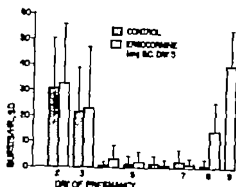


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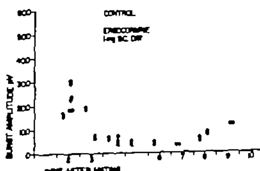


Fig. 5 Daily variations of mean amplitude of bu of electrical activity in individual rats in the middl the uterine horn.

[illegible] I Endorphin content of lumbar CSF [illegible]hile experiencing pain (Control) and after electro-acupuncture (Stimul.).

[illegible]	Age (yrs)	Pain cause	Duration	Analgesia from stimulation	Endorphin content of lumbar CSF			
					Fraction I		Fraction II	
					Control	Stimul.	Control	Stimul.
	45	Posttraumatic neuralgia thigh	4 yrs	Full	<0.4[a]	0.8	[illegible]4	2.2
	59	Mononeuritis of saphenous nerve	1 month	Full	0.5	1.3	2.9	0.9
	49	Herniated disc L4–L5	5 yrs	Partial	0.9	0	0.9	2.4
	48	Operated spinal AV malformation (L5 pain)	21 yrs	Full	<0.4	2.6	0.6	5.[illegible]
	84	Herpes zoster Th 5	8 yrs	Full	<0.4	<0.4	0.6	1.2
	78	Trigeminal neuralgia	16 yrs	Partial	0.4	0.4	4.9	0.6
	75	Trigeminal neuralgia	7 yrs	None	0.4	<0.4	3.8	3.0
	74	Trigeminal neuralgia	12 yrs	Full	<0.4	0.5	2.4	3.5
	64	Trigeminal neuralgia	5 yrs	Full	0.5	0.6	1.3	1.0

[a] [illegible]mol of Met-enkephalin/ml.

[illegible] *al.* 1976). Four to five ml ultrafiltered CSF was run through a Sephadex G10 column [illegible]ted with 0.2 M acetic acid. Two fractions, I and II were collected, lyophilized and assayed [illegible] opiate receptor affinity against tritium-labelled dihydromorphine. A calibration curve [illegible]h authentic Met-enkephalin was run in parallel and the endorphin content was expressed [illegible] picomoles/ml of Metenkephalin.

The two chromatographic fractions (I and II) account for more than 75% of the total [illegible]dorphin activity of the human CSF as measured in the receptor binding assay (Wahlstrom [illegible]*al.* 1976). In patients with no pain and apparently healthy the CSF concentrations of [illegible]se fractions, expressed as picomoles of Met-enkephalin/ml, are 1.4 ± 0.4 (mean ± S.E.) [illegible]nd (I) and 5.2 ± 1.8 pmol/ml (II) respectively (Terenius *et al.* 1976). From the present [illegible]ults (Table 1) it appears that the lumbar CSF content of fraction I is very low in all patients [illegible]le experiencing pain, confirming earlier observations on patients with trigeminal neuralgia [illegible]renius and Wahlstrom 1975 b). No systematic change is seen with fraction II. During [illegible]mulation analgesia a marked rise of endorphin fraction I in lumbar CSF is seen in patients [illegible] 1–4, [illegible]hile this is not the case in the other patients. Again, the content of endorphin [illegible]ction II does not seem related to the analgesic effect.

The relation between CSF endorphin concentration and activity in endorphin systems is [illegible] clear although a direct correlation is likely. If so, the low endorphin fraction I in all [illegible]tients experiencing chronic pain is remarkable. These low levels might be due to an in[illegible]ent hypoactivity in the systems releasing endorphi[illegible] (Terenius and Wahlstrom 1975 b) or [illegible] high consumption of released endorphins. A normalization of the endorphin fraction I [illegible]tent of lumbar CSF with stimulation is seen only in the patients having lumbar pain [illegible] 1–4) and therefore undergoing stimulation of lumbar segments (Andersson *et al.* 1976 b, [illegible]rlsson and Sjölund 1976). On the other hand, patient no. 5, suffering from a post-herpetic [illegible]uralgia engaging the fifth thoracic segment unilaterally and the 4 patients with trigeminal

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Increased Cerebrospinal Fluid Levels of Endorphins after Electro-Acupuncture

By

B SJÖLUND L. TERENIUS and M ERIKSSON

In modern Chinese acupuncture low frequency electrical stimulation of the inserted nee is often used instead of the classical method of manual twirling (Kaada *et al.* 1974 Bo 1974) As confirmed in Western investigations (Andersson *et al* 1973 Chapman *et al.* 19 the pain threshold of healthy volunteers is increased with the procedure. Moreover elec acupuncture performed via surface electrodes has been found to be more effective than via needles (Andersson *et al* 1973) probably because the amount of current passed ca larger and the seemingly necessary muscle twitches in adjacent regions therefore are stro (Andersson *et al* 1976 b). Despite these results, attempts to use acupuncture for the l term relief of chronic pain have been largely unsuccessful (Andersson *et al.* 1976 a, (*et al* 1975) However by modifying the stimulation technique to reinforce muscle cont tions, electro-acupuncture via surface electrodes can give satisfactory relief of chronic (Eriksson and Sjölund 1976)

The mechanism behind acupuncture analgesia remains unclear However naloxo specific opiate antagonist (Martin 1967), counteracts the increase in pain threshold in hea individuals found after classical needle acupuncture (Mayer *et al* 1975) as well as the a gesia from electro-acupuncture in patients with chronic pain (Sjölund and Eriksson 19 A similar effect has recently been reported with mice receiving electro-acupuncture (Po ranz and Chiu 1976) These results suggest the activation of an inhibitory mechanism rele endogenous morphinelike substances (endorphins Hughes *et al* 1975 Terenius and W ström 1975 a) Since it is now possible to determine the concentrations of several endorp in human cerebrospinal fluid (CSF Terenius and Wahlström 1975 b), we have investig whether electro-acupuncture via surface electrodes (Eriksson and Sjölund 1976) change endorphin content of the CSF during the period of analgesia experienced by the patien

Nine patients (Table I) suffering from chronic pain volunteered for the study They un went lumbar puncture twice, once while experiencing pain and without any analgesic r sures during the previous 12–18 h, and once after electro-acupuncture via surface electr (Eriksson and Sjölund 1976) for 45 min Within 30 min after stimulation the CSF collected. The CSF (10 ml/test) was drawn into cooled tubes, frozen within 30 min and st at −20°C for later analysis. Prior to this the CSF was thawed and centrifuged at 1 000 g 10 min The endorphin analysis was performed essentially as described earlier (Tere

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...e Kinesiology Laboratory, University of Jyväskylä, Finland, and Department of Physiology, Gymnastik- och Idrottshögskolan, Stockholm, Sweden

Skeletal Muscle Fibres and Muscle Enzyme Activities in Monozygous and Dizygous Twins of Both Sexes

By

P. V. Komi, J. H. T. Viitasalo, M. Havu, A. Thorstensson, B. Sjödin and J. Karlsson

Received 5 October 1976

Abstract

...P. V., J. H. T. Viitasalo, M. Havu, A. Thorstensson, B. Sjödin and J. Karlsson. *Skeletal muscle fibres and muscle enzyme activities in monozygous and dizygous twins of both sexes.* Acta physiol. scand. 1977. *100*. 385–392.

...nce of the genetic component in determining the interindividual variation observed in skeletal ... fibre composition and enzyme activities was investigated in 31 pairs of male and female monozygous and dizygous (DZ) twins, whose ages ranged in all but one pair (11 years) from 15 to 24 years. Percent ... of slow twitch muscle fibres and activities of Ca^{2+} and Mg^{2+} *stimulated ATPases*, creatine phos-...ase, myokinase, phosphorylase, lactate dehydrogenase (LDH) and distribution of its isozyme LDH-1 ... analyzed in biopsy samples taken from the vastus lateralis muscle. The data disclosed that ... the DZ twins the MZ twins of both sexes had an essentially identical muscle fibre composition. Cal-... of the heritability estimate for this parameter gave the values of 99.5 and 92.8 %, respectively for ... and females. In contrast to the fibre composition presence of significant genetic component was not ... any of the enzyme activities studied. It was concluded that there is predominant genetic in-...e on the skeletal muscle fibre composition in man, and thus also on the potential capacity of the ... to perform work.

...s conducted on monozygous (MZ) and dizygous (DZ) twins have suggested that the ...ic component is of significance for the interindividual variation observed in maximal ...en uptake and muscular power (Klissouras 1971, Komi *et al.* 1973). Other recent reports ... Gollnick *et al.* 1972, Thorstensson 1976) have emphasized the interdependence between ...ility to perform work and skeletal muscle fibre composition.

...seemed, therefore, logical to apply "the twin study concept" as developed by Holzinger ...) to human skeletal muscle and its histochemical and biochemical properties in MZ ... DZ twins to increase our knowledge about the basis and relative significance of the ...type in these respects.

neuralgia were stimulated close to their respective painful areas. They did not exhibit ... change of endorphin fraction I content in lumbar CSF despite the analgesia induced ... cases (Table I) This suggests a local release of endorphins during acupuncture, indic... that the site of action for pain relief by the endorphins is at least partly at the segm... level (*cf* Le Bars *et al* 1975 Duggan *et al* 1976, Yaksh and Rudy 1976). An incre... CSF endorphins might well have been observed in patients no 5 6, 8 and 9 if fluid at ... levels had been analyzed.

This work was supported by the Swedish Medical Research Council. The careful assistance of A ... strom, I Eriksson, A -Ch. Eriksson a d M L. Lindén is gratefully acknowledged.

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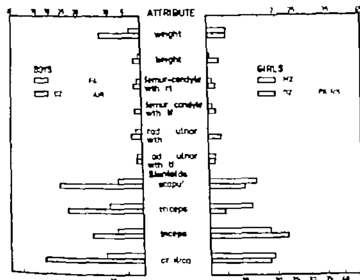
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Mean percent intrapair differences in MZ and DZ twins for anthropometric data in boys (left) and girls (right)

Results

...eneral finding from the different anthropometric measurements was for the males ...he MZ twins demonstrated smaller intrapair variations than their DZ counterparts ...1). This pattern was not, however as consistent for the females.

...letal muscle fibre distribution (ST %) was almost identical within MZ in contrast to ...twin pairs (Fig. 2 and 3). The H_{est} calculations (Table II) for this attribute gave the ...ing results 99.5% (males), 92.2 (females), and 96.5% (males females) indicating ...ence of a strong genetic component. The ST % values for the MZ pairs were within ...ror of measurements, while those of the DZ pairs were randomly scattered.

...contrast to the skeletal muscle fibre distribution none of the enzyme activities measured ...strated any significant variability difference between MZ and DZ populations. ...to be pointed out, however that LDH activity and distribution of LDH 1 isozyme ...more narrow in female MZ twins, whereas the variability was greater in female DZ ... (Fig. 4) and similar as in males irrespective type of zygosity. However the F-ratio ...) was not high enough to reach the required significance level of p 0.05

Discussion

...most important finding in this study was the strong heritability estimate (H_{est}) observed ...he ST fibre distribution in all subjects.

...mputation of H_{est} value assumes that the environmental influences are comparable ...g the twin pairs (Klissouras 1971). This was carefully taken into account in previous

TABLE I Twin pairs distributed according to age and zygosity

Zygosity	Boys age (yrs)											Total pairs	Girls age (yrs)										Total pairs
	11	15	16	17	18	19	20	21	22	23	24		15	16	17	18	19	20	21	22	23	24	
MZ	1	2	1	2	–	–	2	–	1	–	–	9	1	1	–	1	–			1	1	1	6
DZ		2		1		3		2	–	–	1	11		–	1	1	1	1	1				5
Total pairs	1	4	3	3		3	2	2	1	–	1	20	1	1	1	2	1	1	1	1	1	1	11

Methods

Subjects for the study were obtained through the Population Register of Finland. This register provid the names and addresses of families with two or more children born on the same day in the city or su rounding communities of Jyväskylä between the years of 1950 and 1953 In addition one pair as obtained from T rku and two pairs from Stockholm. The final sample was composed of 20 male (9 MZ and 11 DZ) nd 11 female (6 MZ nd 5 DZ) twin pairs (Table I). Initial determination of zygosity as performed by subjective observat on of physical appearance. Certainty of genetic identity or non-identity thin the pair was increased by serological analyses. Venous samples of 7 t 8 ml of clotted blood ere collected from each subject and further analysed according to instructions of Race and Sanger (1968) and Giblett (1969). Antisera for the determination of the following red cell a tigens were used A A BO MNS_s, $Rh(CC^wDE_{ce})$ P Lu^a K_p, Fy^a and Fy^b and Jk The serum was separated and frozen for later n estigation. The following proteins and enzymes were determined Haptoglobin group specific substance, acid phosphatase (E.C 3 1.3 2), myokinase (MK, E.C.2.7 4 3), and phosphoglucomutase (E.C.2.7.5.1). I the 16 cases of observed dizygosity disco dance was bserved in more tha five antigens or serum proteins.

A thropometric measurement In addition to recordings of body weight and height the following an thropometric measurements were performed femurcondyle and radioulnar widths and skinfolds of sub scapular triceps brachii, biceps brach i, and supra iliac area (Durnin and Rahaman 1967). For correlation analysis, fat free body weight was also estimated utilizing the method of von Dobeln (1959).

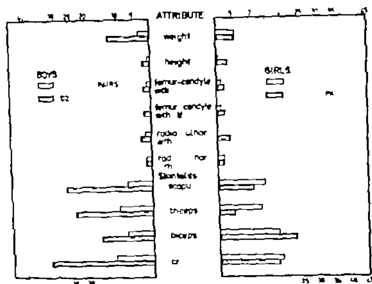
Sk letal muscle fibre composition Two muscle biopsy samples were taken from the vastus lateralis muscle with a biopsy needle as described by Bergstrom (1962). In this method the skin and underlying fascia were locally anaesthetized and a small 5 mm wide cut was made so that the biopsy needle could be inserted into the muscle. The site and depth of nsertion were standardized for all subjects The first biopsy sample was used f classification of muscle f bres int the two mai f bre types, fast tw tch (FT) and slow twitch (ST) (Gollnick t al. 1972) by staining for ATPase as instructed by Padykula and Herman (1955). The error of measurement was calculated for do ble biopsies taken d fferent d ys from a number of subjects.

Muscle enzymes. The second biopsy sample was used for determination of enzyme activities. The enzymes investigated were Mg^{2} stimulated ATPase, creat ne phosphok nase (CPK) and myokinase (MK) according to Thorstensson (1976) lactate dehydrogenase (LDH) according to Sjödin (1976) phosphorylase (Plase), hexokinase (HK), and Ca^{2} stimulated ATPase (Lowry and Passonneau 1972). Distribution of the heart muscle specific LDH isozyme, LDH 1 was determined in the muscle biopsy specimens as described by Karlsson *et al.* (1974).

Statistical analysis In add tion to the rdinary statistical procedures employed to calculate the mean, standard deviation (SD) and correlation coefficient ( ), the single analysis of variance was used to test the significance of differences between the mean intrapa variances of the two twin types. If the ariance ratio (F) was significant at the 5 level of probability the computation of the heritability estimate as done as originally described by Holzinger (1979)

$$H_{est} \frac{S^2DZ \quad S^2MZ}{S^2DZ \quad S^2e} \qquad (1)$$

where S^2MZ and S^2DZ denote intrapair variability of attribut in MZ d DZ twi s, respectively and S^2e signifies the variance due to experimental error Formulas gi en by Partanen t al (1966) were used for computation of S^2DZ and S^2MZ. H_{est} gives an estimation of the relative cont bution of the genetic component in explaining the ariability in given tt bute.



Mean percent intrapair differences in MZ and DZ twins for anthropometric data in boys (left) s (right).

Results

...neral finding from the different anthropometric measurements was for the males ...e MZ twins demonstrated smaller intrapair variations than their DZ counterparts ...). This pattern was not, however as consistent for the females.

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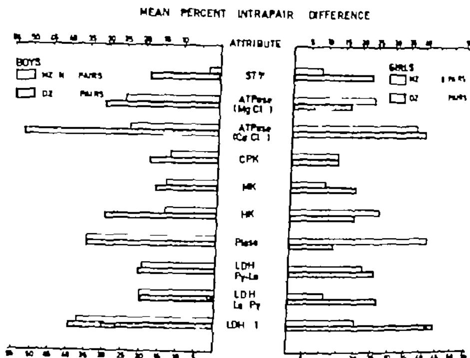
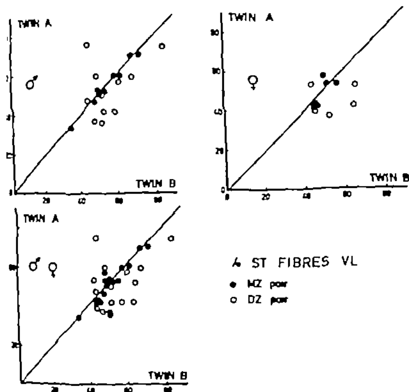


Fig. 2. Mean percent intrapair difference in MZ and DZ twins for the distribution of slow twitch fibres (ST %) and for activities of the different skeletal muscle enzymes.

study on a similar population in Finland (Komi *et al* 1973). However no special control of the socio-economic, health or physical activity status was made between the pairs or groups in the present study. Although these environmental influences probably have been only minor as indicated by Komi *et al* (1973) they still might be the cause of the finding of no genetic component in muscle enzyme activities as will be discussed later.

The needle biopsy technique and the reproducibility of the results obtained in the specimen might be questioned. Previous methodological studies on metabolites (Karlsson 1971, Harris *et al* 1974) fibre distribution (Piehl 1974) and enzyme activities (Gollnick *et al* 1974) have demonstrated a good reproducibility in the investigated muscle (usually m. vastus lateralis) of the technique and methods applied in the present study. A question may also be raised to what extent m. vastus lateralis is representative for the entire skeletal muscle pool and whether findings in that particular muscle can be generalized to other muscle groups. Correlation coefficients for percent ST fibre distribution between e.g. vastus lateralis and deltoideus muscles (r = in the order of 0.60 to 0.80) indicate that a relationship exists (Edström and Nyström 1969 Komi *et al* 1977).

From animal studies we know that a motoneuron of a motor unit innervates a both histochemically (e.g. Brandstater and Lambert 1969) and physiologically (e.g. Burke *et al* 1971) uniform type of muscle fibres. There is, to our understanding, no reason to believe that the situation would be different in man. This means that by determining muscle fibre composition, not only the metabolic profile but even the neuro-motoric control of the muscle is indicated to a certain extent. Empirical evidence in man for this has already been published as relationship between V_{O_2} max as well as type of sport activity and muscle fibre



1. Intrapair comparison of slow twitch fibre distribution of m. vastus lateralis in MZ and DZ twins.

ribution in top athletes (Costill *et al.* 1976, Forsberg *et al.* 1976, Rusko *et al.* 1976, ph *et al.* 1977). Moreover the significance of fast twitch (FT) fibres for muscle strength elopment has recently been documented experimentally in human skeletal muscle orstensson 1976), which confirmed earlier postulations concerning the biological implica- of FT fibres in man (Gollnick *et al.* 1972).

The high H_{est} value found in the present study for ST fibre distribution in the vastus ralis muscle explains why many investigations in humans have failed to observe any nges in the ST fibre distribution with either endurance training (Gollnick *et al.* 1973) strength training (Thorstensson *et al.* 1975 1976 a, b).

BLE II Estimates of variance within monozygous (S^2MZ) and dizygous twins (S^2DZ), variance of error of measurement (S^2e), and heritability of variation (H_{est}) of fibre composition of vastus lateralis muscle in twins of both sexes

	S^2e	S^2MZ	S^2DZ	H_{est}
ales	2.15	2.24	94.62	99.5
males	2.15	10.55	118.56	92.8
ales and females	2.15	5.31	98.52	96.7

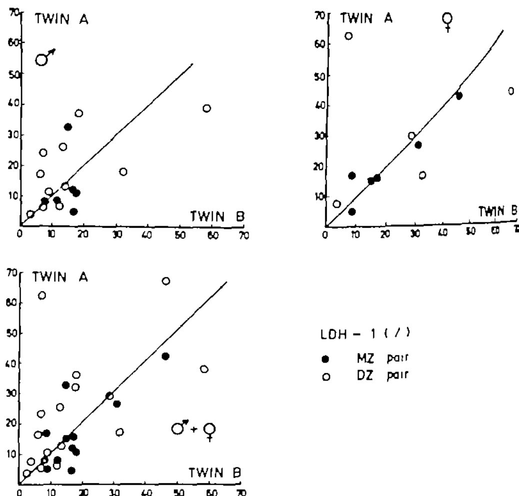


Fig. 4 Intrapair compariso of LDH 1 isozyme distribution in MZ and DZ twins.

As regards muscle enzyme activities Sjödin *et al* (1976) have demonstrated that environmental factors such as endurance training affect not only LDH activity but also LDH isozyme distribution In the present material the MZ females demonstrated smaller intrapair variations in LDH activity as well as in LDH isozyme distribution than their male counterparts Most probably the males may be under more influence of environmental factors such as daily physical activity This discrepancy might further support the suggestion that genetic factors basically are of some significance also for muscle enzyme activities.

In this connection it is interesting to know whether the magnitude of training effects on the circulatory apparatus in one way or the other is related to muscle fibre population rather than activity level thus resulting in the correlation reported for $\dot{V}_{O_2}$ max and percent ST fibres (Rusko *et al* 1976, Bergh *et al* 1977) in habitually physically active or homogeneously trained individuals. If that is the case it has to be suggested that also the type and magnitude of adaptive response might be influenced by a genetic predisposition

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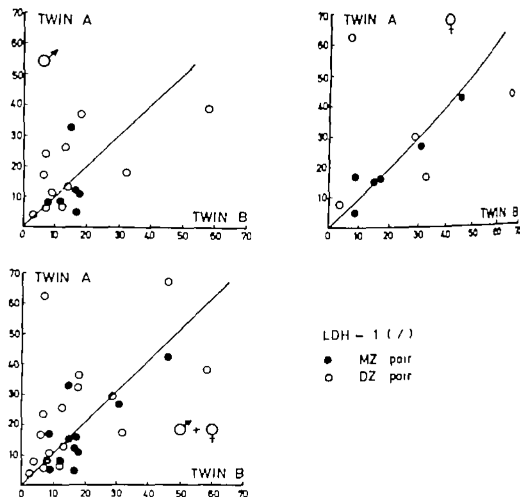


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From the Zoophysiological Laboratory B, August Krogh Institute,
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Cochlear Potentials of the Pigeon Inner Ear Recorded with Microelectrodes

By

Finn Ove Jørgensen

Received 21 October 1976

Abstract

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CM-intensity function (i.e. the relation between the amplitude of the cochlear potential (CM) and the sound pressure level) of the avian ear has previously been shown to deviate from the mammalian ear with respect to the slope of the linear part of the function. The lower slope values found in the perilymphatic space of the avian ear have been interpreted to indicate a damping influence of the tegmentum vasculosum on the mechanical vibration of the basilar membrane. In this study the CM-intensity function has been measured on either side of the tegmentum vasculosum of the pigeon ear using microelectrode technique. The slope of the linear part of the CM-intensity function in scala vestibuli was found to be a function of the sound frequency approaching unity at higher frequencies. In ductus cochlearis the slope of the linear part of the CM-intensity function was close to unity at most frequencies except the best frequency for the electrode position. At this frequency (3–4 kHz) the slope value was low. These findings from the ductus cochlearis are similar to findings in scala media of the mammalian ear. Thus the influence of tegmentum vasculosum on CM in scala vestibuli may be due to passive electrical properties of the tissue and not to the mechanical vibration of the basilar membrane.

The relation between the amplitude of the cochlear microphonic potential (CM) and the sound pressure level—the CM intensity function—in the avian ear has previously been obtained from recordings in the perilymphatic space or at the round window membrane (Wever and Bray 1936, Bleeker and de Vries 1948, Schwartzkopff 1960, Necker 1970). At moderate sound pressure level the relation between CM and the sound pressure could be described by a power function with power values between 0.35–0.5. This is considerably less than found in the mammalian ear where the power value was close to unity at a similar recording location. The low power value in the avian ear has been interpreted to indicate a damping influence of tegmentum vasculosum upon the mechanical movement of the basilar membrane (Schwartzkopff 1968).

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Fig. 1 Drawings of the pigeon cochlea with the electrode position indicated (A) and of a cross section of cochlea at the approximate position of electrode placement (B). A The drawing of the cochlea is ba upon a photograph of a preparation of the right cochlea of pigeon The electrode a placed n the coch apically to the oval window A part of the horizontal semicircular canal is shown B The cross section pigeon cochlea shows the posit on of the three scalae scala vestibuli (sc vest), ductus cochlearis (d c) recessus scala tympani (sc.tymp) Tegment m vasculosum separates scala vest buli from ductus cochlear and pars basilaris separates ductus cochlearis from scala tympani.

the cochlea influences markedly the cochlear microphonic potential recorded distant fro the place of generation (Weiss, Peake and Sohmer 1971)

In order to elucidate further the rôle of the tegmentum vasculosum on CM, the CI intensity functions have been reinvestigated at frequencies appropriate for the recordi location on either side of the tegmentum vasculosum.

Methods

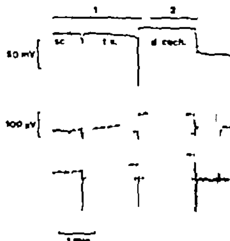
The experiments were performed on pigeons (*Columbia li ia*) weighing 300 t 400 g. The experiment procedure used in this study ha been described previously (Jorgensen 1975).

Results

The electrical potential profile

An electrode correctly placed in scala vestibuli will upon advancement pass through teg mentum vasculosum and ductus cochlearis to scala tympani (cf Fig. 1) The actual route t the electrode is dependent upon the angle of the electrode relative to the cochlea An examp of the dc-potential variation and the corresponding ac potential elicited by sound stimul tion, when the electrode is moved transversely to the longitudinal axis of the cochlea, shown in Fig. 2.

The solid horizontal lines above the recording indicate the movement of the electrod (4 steps/s = 3.2 μm/s) The upper trace shows the dc recording. The initial dc level remaine stable, as the electrode was advanced 160 μm. Upon further advancement a positive dc-lev is reached The two dc levels are separated by negative potential deflections. The positi



The electrical potential profile of the pigeon ... recorded with ... microelectrode moved from vestibuli towards pars basilaris. The electrode advanced with constant step frequency indicated ... the horizontal lines marked 1 and 2. The upper ... shows the variation in dc potential along the ... track. The assumed positions of the elec... tip ... the cochlea are indicated (sc ... scala vestibuli, t ... tegmentum vasculosum, d coch. ... ductus cochlearis, and p b ... pars basilaris). The ... changes in the amplitude of the ... fundamental frequency of the cochlear microphonic ... (CM) elicited by 4 kHz sound pulses are ... in the middle trace. The lower trace shows the ... of the phase sensitive detector. Note that ... the input p b the ... voltage from the phase ... detector becomes zero indicating ... phase ... of 90° for CM

...ential probably reflects the electrical potential of an extracellular space. When the electrode was moved further a negative potential of 40 mV (relative to scala vestibuli) was recorded. The electrode advancement was stopped as soon as the negative potential was recorded, and the potential remained stable for 3 min until the electrode was withdrawn. The second trace shows the amplitude of the fundamental frequency of the ac-cochlear potential (cochlear microphonic potential (CM)) when the ear was stimulated by sound-pulses at 4 kHz (SPL 70 dB). The amplitude of CM is seen to increase simultaneously with recording of the positive potential. The amplitude of CM remained practically constant until the negative potential was recorded. This caused a reduction of the amplitude o CM. The lower trace shows the output from a phase sensitive detector when the electrical signal containing CM was fed into the amplifier together with the reference signal (the driving voltage of the loudspeaker). It indicates that only a minor change in phase of CM took place by moving the electrode from scala vestibuli until the positive potential was reached. In contrast, a phase shift of 90 relativ to ductus cochlearis was found when the subsequent negative potential was recorded.

This example of a single traverse by an electrode is not completely representative. Particularly the potential changes before the positive potential was recorded were often found to be more complex. Also potential changes beyond the positive potential ary considerably. But the example serves to illustrate the criteria used for determin ng that the electrode tip was located in ductus cochlearis. The criteria are analogous to those used for the mammalian ear (Bekesy ... Peake and Weiss 1971). Thus the electrode tip is assumed to be in ductus cochlearis, when the amplitude of CM increases instantaneously with recording of a positive potential and with only a minor phase shift of CM associated (0-30°). Furthermore this positiv potential should be sensitive to anoxia.

The endolymphatic potential (EP)

The electrical potential of the ductus cochlearis (the endolymphatic potential (EP)) relativ to scala vestibuli was found to be 11 ± 3 mV (SD for ... 58).

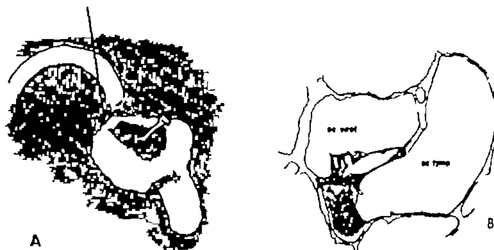


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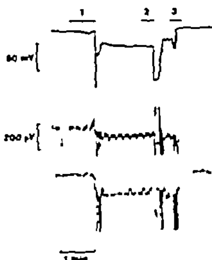
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4 Amplitude enlargement of CM in pars basilaris. The electrode tip was advanced from ductus cochlearis towards pars basilaris (trace). A the same potential was recorded the electrode advancement was arrested. The amplitude of CM to the sound pulses was reduced (trace b) and a sink of 180° was observed (trace). Upon withdrawal of the electrode tip (2) a negative potential was recorded together with an enlargement of the amplitude of CM. The enlargement was maintained as long as the negative potential was stable. A slight change in the phase angle of CM was found. 3 the electrode tip was withdrawn to ductus cochlearis.

...nt of the amplitude of CM recorded in pars basilaris. The enlargement of CM was in this ... recorded upon withdrawal of the electrode tip from pars basilaris. The electrode was ...pped when the large negative potential was recorded. (Since the displacement direction ... the electrode was reversed the horizontal bar does not indicate the distance which the ...ctrode was moved. The length of the horizontal bar corresponds to approximately 70 ...ps, which is the number required for the stepmotor to catch up with the slip in the me...chanical system.)

...nsity functions of CM in the two scalae

The intensity functions of CM were determined in both scala vestibuli and in ductus cochlearis. Fig. 5 (left) shows an intensity function obtained in scala vestibuli. The logarithm of the amplitude of CM at different frequencies is plotted as a function of the sound pressure level (dB). The relationship between CM and SPL was linear at moderate sound pressure levels but departed from linearity at 80 to 90 dB (SPL) for frequencies of 4 kHz and less. At 5 and 6 kHz, however, CM did not depart from linearity at the SPL used. The slope of the straight line segment (*i.e.* the change in amplitude of CM for 20 dB change in SPL) ...ed between 0.35 and 1.0. The pattern of slope changes in five experiments was consistent with that seen in this example, all of them showing an increase as the frequency of the ...mulus sound is increased.

In ductus cochlearis the CM-intensity functions showed different properties (Fig. 5 ...ht). The slope of the straight line segment obtained at lower frequencies (1 and 2 kHz) was ...creased (relative to scala vestibuli), whereas the slope obtained at 3 kHz remained very ...w. Thus a plot of the slope value as a function of frequency would give a minimum value at the specific frequency. These findings are representative for 9 experiments with respect to the high value of the slope of the straight line segment (0.8–1.0) found at frequencies ...rent from the best frequency. In four cochleae where CM at 4 kHz was the best frequency

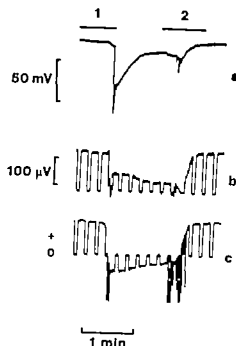


Fig. 3 Amplitude reduction of CM in pars basilaris upper trace (a) shows the dc potential changes as the trode was moved from ductus cochlearis (positive pote to pars basilaris (1). The advancement of the electrod was arrested, when the negative potential was recorded the electrode tip was withdrawn. The amplitude of to 3 kHz sound pulses was reduced as the negative pote was recorded (trace b), and a phase shift of 180° was obs (trace c).

In a preliminary study using 6 pigeons the magnitude of the endolymphatic potential not affected by elevation of the oxygen content of the inspired gas mixture from 21% 30%

The endolymphatic potential was stable for several hours. The stability and the magni were the same when larger electrodes were introduced into the endolymphatic space. Tl electrodes were used in a few cases when the waveform of CM during anoxia was exami (Jørgensen 1975) Repenetration of the endolymphatic space usually gave smaller posi potentials

Recordings from pars basilaris

Negative potentials which followed the positive potentials as the electrode was advan could, according to the anatomy of the cochlea, either arise from penetration into anot lobe of the tegmentum vasculosum or reflect the potentials of cellular compartments of pars basilaris. The electrode was believed to be in pars basilaris, when negative potent were recorded simultaneously with recordings of CM which showed large phase sh (Békésy 1952, Tasaki, Davis and Eldredge 1954 Weiss, Peake and Sohmer 1971).

Negative potentials which met these criteria varied between 50 and 100 mV The potenti were quickly reduced towards zero (5–20 s) suggesting that they were of cellular origin. T amplitude of CM was usually found to be reduced but the associated phase shift was la (170°–180° relative to ductus cochlearis) An example of the events which take place wh an electrode is moved into pars basilaris from ductus cochlearis is shown in Fig. 3

Occasionally the amplitude of CM recorded in papilla basilaris was larger than C recorded in ductus cochlearis. The amplitude of CM in these cases remained large as long the negative electrical dc-potential was unaltered. Fig. 4 shows an example of an enlarg

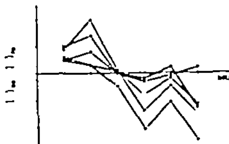


Fig. 7 Amplitude relation between CM recorded in scala vestibuli and in ductus cochlearis. The difference between the ratio CM ... CM obtained at 90 dB and the ratio obtained at 70 dB (R) is plotted as a function of the sound frequency. Each curve represents one experiment.

the ratio at higher frequencies is the opposite, although the change in magnitude of the ratio is small. Only at 3 kHz CM_{dc}/CM_{sv} is unaffected by changes in the sound pressure level.

Although the example shown in Fig. 6 is consistent with the results of five experiments, the absolute values of the magnitude of the ratio showed large variations. Therefore, in order to show the effect of elevated sound pressure level at the different frequencies, a simple average of the results was not appropriate. In Fig. 7 the difference between the ratio CM_{dc}/CM_{sv} obtained at 90 and 70 dB is plotted as a function of the stimulus frequency. Each curve represents one experiment. This figure shows that the ratio between the CM amplitudes on the two sides of the tegmentum vasculosum is a complex function of frequency and the sound pressure level. Only CM at 3 kHz is not influenced by the sound pressure level.

CM frequency response curves

The data from the CM intensity functions obtained in ductus cochlearis can be used to describe the CM frequency response curves for one position of the electrode. In Fig. 8 the amplitude of CM is plotted as a function of the stimulus frequency. Points of equal sound pressure level are connected. Five frequency response curves at different SPL are shown. The curve obtained at 50 dB is narrowly tuned with 3 kHz as the best frequency. The curve form is shown to be a function of the sound pressure level. Both the width of the tuning curves and the slope values are changed by elevated sound pressure. The relative bandwidth (bandwidth at half power divided by the best frequency) changes from 0.1 at 50 dB to 0.6 at 90 dB. The low frequency slope changes from 30 dB/octave to 6 dB/octave. The high fre

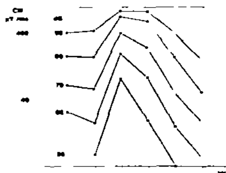


Fig. 8 CM frequency response curves in ductus cochlearis. The amplitude of CM is plotted as a function of the stimulus frequency at constant sound pressure level. The data are taken from the CM intensity functions shown in Fig. 5 (right). The points for 2 and 5 kHz at 50 dB are determined by linear extrapolation of the straight line segment of the intensity functions.

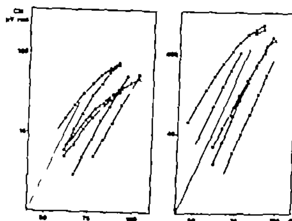


Fig. 5 CM intensity functions in scala estibuli and in ductus cochlearis. The amplitude of CM to … of different frequencies is plotted as a function of the sound pressure level (SPL). The CM int… functions were first obtained in scala vestibuli (left) and the electrode tip was then advanced to d… cochlearis (right) just bove the pars basilaris. The thin line drawn in each figure has a slope of … when CM is related to the sound pressure In this case the amplitude of CM increases ten times for a… fold increase n the sound pressure This corresponds to a 20 dB increase i sound pressure (SPL) since $SPL = 20 \log p/p_0$ dB, where p is the sound pressure and p_0 is the reference sound pre… (□ = 1 kHz, Δ = 2 kHz, ● = 3 kHz, ▲ = 4 kHz, ○ = 5 kHz, ■ = 6 kHz.)

(largest amplitude of CM at 70 dB) the lines of CM both at 3 and 4 kHz exhibited low … values (0.3–0 5)

Relation between CM in ductus cochlearis and CM in scala vestibuli

CM recorded in scala media in the mammalian ear is larger than CM recorded in s… vestibuli (1–5 dB) but the enlargement is independent of the sound frequencies and … sound pressure level (Weiss, Peake and Sohmer 1971) In the pigeon the corresponding … largement of CM recorded in ductus cochlearis compared to CM recorded in scala vesti… was found to be different

The ratio CM_{dc}/CM_{sv} is plotted in Fig. 6 as a function of stimulus frequency for … different sound pressure levels (60 and 90 dB) The figure shows that both stimulus freque… and sound pressure level have an influence upon the ratio At low frequencies (3 k… and at low sound pressure level the ratio is small. The ratio increases as the frequency is … creased reaching a maximum at 4 kHz. At elevated sound pressure level CM_{dc}/CM… becomes larger at the lower frequencies, because CM_{dc} increases more rapidly with incre… in sound pressure level than does CM_{sv} The effect of elevated sound pressure level up…

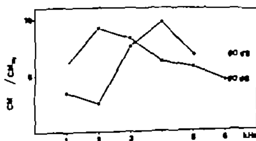


Fig. 6 Amplitude relation between CM recorded … scala estibuli and in ductus cochlearis. The ra… between CM recorded in ductus cochlearis (CM… nd CM recorded in scala vestibuli (CM_{sv}) is plo… ed as a function of the sound frequency This relatio… is plotted at tw sound pressure levels, 90 dB (f… ed circles) and 60 dB (open circles)

& Ward 1968, Hoerubla, Strelioff and Ward 1973 Dallos 1973 Cheatham and Ferraro 79)

The present finding, that CM intensity functions obtained in ductus cochlearis (Fig. 5 b) exhibited slope values close to unity at most frequencies except at the best frequency is in accordance with the findings in the mammalian cochlea. The difficulties in getting to more distal parts of the basilar membrane prevented a further examination of the illustrated pattern in relation to place along the basilar membrane.

The apparent difference in CM intensity function between the mammalian and the avian t. ben recorded in the perilymphatic space, was interpreted to indicate the damping of tegmentum vasculosum upon the mechanical vibration of the basilar membrane (Schwartzkopff 1968). This interpretation does not seem valid in view of the present finding CM intensity functions obtained in ductus cochlearis exhibit normal slope values at frequencies.

An influence of tegmentum vasculosum on CM recorded in scala vestibuli may be inferred from Fig. 6 and Fig. 7 Here the ratio of CM recorded on either side of tegmentum vasculosum is shown to be a function of the frequency and the sound pressure level. A similar effect of frequency and sound pressure level on the ratio of CM recorded on either side of Reissner's membrane in cat was not found (Weiss, Peake and Sohmer 1971) But the ratio of CM recorded in scala media and scala tympani was found to be a function of both frequency and the sound pressure level (Weiss, Peake and Sohmer 1971). The qualitative similarity between CM_{sv}/CM_{sm} in the pigeon ear and CM_{sm}/CM_{st} in the ear of the cat may indicate an unspecific, but complicated effect of the tissue separating the two recording positions. The effect is probably due to the passive electrical properties of tegmentum vasculosum of the pigeon and the basilar membrane complex in the cat.

A relation between the mechanical vibration of a restricted area of the basilar membrane and CM recorded with differential electrodes in the perilymphatic space has been found by Wilson (1973) comparing CM data with mechanical data from Wilson and Johnstone (1972). The comparison suggests that CM at low SPL may form a good representation of the extent of the basilar membrane vibration. A similar procedure cannot be followed in the pigeon ear, since CM data from ductus cochlearis at the positions along the basilar membrane where mechanical tuning curves have been determined (von Békésy 1944) cannot be obtained due to anatomical restraints. The CM frequency response curve at 50 dB SPL compared to these mechanical tuning curves obtained at a lower frequency and at higher sound pressure level shows considerably sharper tuning. The series of mechanical tuning curves of the hen basilar membrane indicate, however *that the mechanical tuning sharpens as the columella footplate is approached.*

The CM frequency response curves obtained in the pigeon (Fig. 8) are like CM response curves from the guinea pig a function of the sound pressure level (Dallos 1973, Fig. 4). The high frequency slope of the frequency response curve in guinea pig was found to be 20–40 dB/octave compared to 40 dB/octave in the pigeon both sets of data obtained at low sound pressure level. When the CM frequency response curves are referred to constant velocity of the stapes and the columella (Saunders and Johnstone 1972), the guinea pig curve is hardly affected whereas the high frequency slope of the pigeon curve is reduced by 10 dB/octave

quency slope changes from 40 dB/octave to 15 dB/octave. 3 kHz has been regarded as the best frequency in these calculations although it appears that the best frequency increases at high sound pressure level

The example is representative of the effect of sound pressure level on the form of the CM frequency response curves. Usually a change in the best frequency was found between 80 and 90 dB In these experiments the CM frequency response curve at 70 dB was more narrowly tuned than in experiments where the change in the best frequency took place at 70–80 dB

In 9 experiments the range of the relative bandwith at 70 dB (SPL) was 0.2 to 0.5. The range of the high frequency slope was 11 to 20 dB/octave and the low frequency slope varied between 7 and 16 dB/octave. The best frequency was either 3 or 4 kHz.

Discussion

The magnitude of the endolymphatic potential determined in these experiments (11 ± 3 mV) is in general agreement with previous measurements in the pigeon ear by Schmidt and Fernandez (1962) They reported an endolymphatic potential of 8–9 mV recorded with Ringer-filled pipettes. In the starling and the sparrow the endolymphatic potential was found to be 15 mV (Necker 1970). He suggested that the higher value of the endolymphatic potential compared to the value determined by Schmidt and Fernandez might reflect species differences between songbirds and the pigeon. The measurement of the endolymphatic potential reported here does not support this suggestion

The large negative potentials recorded when the electrode was moved further than the endolymphatic space probably reflect the potentials of cellular compartments of the papilla basilaris. Even if the electrode movement was arrested when the negative potential was recorded, the potential was stable only for a short period of time. The large change in phase of CM which accompanied the transition (170 –180° relative to ductus cochlearis) suggests that the membrane area responsible for the generation of CM was penetrated.

The CM intensity function obtained in scala vestibuli showed a tendency for the slope of the straight line segment to increase with increasing frequency of the sound stimulus. The lowest of the present slope values are in agreement with previous investigations of the pigeon ear (Wever and Bray 1936, Bleeker and de Vries 1948) A similar relationship between CM and the sound pressure level was found when CM was elicited by click stimulus (Schwartzkopff 1960, Necker 1970). The CM intensity functions of the avian ear is in contrast to similar recordings of CM in the basal turn and from the round window membrane of the mammalian cochlea. Here the slope of the CM intensity function was found to be close to unity (Wever and Lawrence 1954)

A more general concept of the relationship between CM and the sound pressure level in the mammalian ear has been established by recordings of CM at different positions along the basilar membrane. CM at all frequencies showed departure from linearity at a given sound pressure level but the early onset of nonlinearity of the CM intensity function was frequency-dependent for a given electrode position, in such a way that CM close to the best frequency showed the first sign of nonlinearity (Tasaki, Davis and Legouix 1952, Honrubia

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(3–6 kHz) Provided that the mechanical vibration of corresponding areas of the basi membrane of guinea pig and pigeon is similar the similarity between the CM frequer response curves may thus indicate that CM recorded with a microelectrode in ductus co learis may form a good representation of the vibration of the basilar membrane.

In conclusion the electrical potential of the endolymphatic space has been measured a was found to be +11 mV ± 3 mV CM intensity functions were obtained in scala vesti and in ductus cochlearis. The former was in agreement with previous findings in the avi ear whereas the latter has not been measured previously CM intensity functions in the duct cochlearis were found to be similar to CM functions obtained in the mammalian ear wh recorded from scala media or using differential electrode technique.

It is suggested that the influence of tegmentum vasculosum on CM recorded in sc vestibuli is due to passive electrical properties of the tissue and not to a mechanical damp of the basilar membrane.

Additional note

Since the completion of this manuscript, evidence has appeared (Pierson and Dallos 19 which supports and extends the relation, described in this paper between CM and the sou pressure level in the perilymphatic space of the pigeon ear It was demonstrated that the C intensity function (the input-output relation) showed an initial slope of one at all frequenc at very low sound pressure level. CM in these experiments was recorded with differen electrodes placed in scala vestibuli and scala tympani proper Departure from linearity w first observed at the best frequency for the electrode position but as the sound pressure le increased the region of nonlinearity spread to lower frequencies.

The results of Pierson and Dallos (1976) may indicate that the nonlinearity of CM frequencies equal to and lower than the best frequency found in the present experiments m be explained by the difference in the lowest sound pressure level used in the two investi tions. The finding in this paper that the CM intensity functions change when CM is recor in ductus cochlearis indicates that the origin of the nonlinearity observed in scala vestib and scala tympani does not arise from the generation of CM alone but may be influen by electrical properties of the tissue separating the recording electrode from the site generation of CM

It is a pleasure to express my thanks t the scientific and technical staff of the *Zoophysiological Labora* B for the support given to me throughout the course of this project.

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TABLE II. Mean of three measurements of red cell velocity in arterioles during each period of low oxygen and room oxygen tension

[illegible]	Mean flow velocity ($\bar{V}$cl)			Relative[a] velocity		
	Low O_2 mm sec^{-1}	Room O_2 mm sec^{-1}	Low O_2 mm sec^{-1}			
5	0.77	[illegible]	[illegible]	3	1	2
6	2.50	0.47	—	2	1	
5	0.55	0.68	0.71	1	2	3
4	0.44	0.23	0.43	3	1	2
3	0.97	0.71	1.19		1	3
4	0.75	0.31	0.53	3	1	2
3	2.99	1.88	—	2	1	
4	1.90	1.04	1.25	3	1	2
4	3.71	2.50	—		1	
4	0.85	1.36	1.05	1	3	2
4	2.29	2.82	3.44	1	2	3
5	1.34	1.00	1.22	3	1	2
2	2.25	0.82	1.94	3	1	2
3	0.66	0.52	0.48	3	2	1
4	1.50	1.06	1.58		1	3
3	1.64	3.3	1.72	1	3	
1	1.36	0.38	0.41	3	1	2
4	1.88	1.85	1.04	3	2	1
3	1.71	0.83	1.79	2	1	3
2	1.25	1.12	1.02	3	2	1
3	1.80	0.94	0.54	3	2	1
2	1.35	1.93	1.31	2	3	1
3	4.17	3.13	—	2	1	
2	[illegible]	0.97	1.07	3	1	2
3	2.51	1.34	1.04	3	2	1
1	1.70	1.35	1.61	3	1	2
1	0.86	1.51	1.23	1	3	2
2	4.69	5.00	3.13		3	1
2	7.34	6.88	—	2	1	
3	1.94	1.76	1.76	2	1	1
2	4.57	2.82	3.13	3	1	2
1	3.38	3.58	2.31	2	3	1
1	4.42	4.25	4.94	2	1	3
2	1.94	1.56	1.29	3	2	1
1	6.25	7.07	—	1	2	
1	3.94	2.49	4.38	2	1	3
1	5.19	3.75	2.19	3	2	1

[a] Values listed in this column are a qualitative comparison of flow velocity in the low, room and low oxygen periods. The number 3 indicates the greatest magnitude and the number 1 indicates the lowest magnitude.

Spontaneous vasomotion of arterioles was seen in most preparations and arteriolar diameter never exceeded 2/3 of the diameter of adjacent venules.

Table I and II list mean diameters and mean flow velocities of 37 arteriolar vessels which was exposed to a low oxygen tension superfusant (PO 30 mmHg) and high or room oxygen tension superfusant ($PO_2 > 115$ mmHg). 31 arteriolar vessels were again exposed to the low oxygen tension superfusant. A qualitative comparison of mean diameters and mean RBC velocities during the 3 periods is presented in Table I and II. From the individual mean diameter and mean flow velocity values the volume flow values for each single vessel were

determined with respect to small vessel diameter this value was used in calculating RBC velocity in arterioles, even though it may not be strictly applicable in these vessels. Due to the wide range of the val the statistical analysis by sign test was performed on the results of qualitative comparisons of : duri g the 3 different oxygen periods.

Results

2 of the 10 hamster preparations showed unsatisfactory blood flow (No 3 and 7) and w not used for measurements. One preparation (No 4) was accidently damaged follow the room oxygen period but values from the first low and room oxygen period were includ

TABLE I Mean of three measurements of arteriolar diameter during each period of low oxygen and r oxygen tension.

Hamster	Vessel	Mean diameters			Relative[a] diameter		
		Low O_2 Diam. μm	Room O_2 Diam. μm	Low O_2 Diam. μm			
9	5	4 2	4 4	4.5	1	2	3
4	6	5.2	2.6	—	2	1	
10	5	5.5	4.8	5.5	2	1	2
9	4	5 9	5 8	5.5	3	2	1
1	3	6.3	7.0	6.3	1		1
1	4	6.3	7 0	8.5	1	2	3
4	5	6 9	6.1	—	2	1	
5	4	6.9	6.9	6.9	1	1	1
4	4	7.5	5.2	—	2	1	
10	4	7.5	5 8	7.3	3	1	2
2	4	7 5	6.1	7 8	2	1	3
6	5	7.8	5.2	10 4	2	1	3
1	2	8.5	9 9	8.5	1	2	1
9	3	8.5	7.2	6.7	3	2	1
8	4	8 6	5.[illegible]	11 0	2	1	3
10	3	12.0	8.7	10.8	3	1	2
1	1	12.7	19.0	25 4	1	2	3
6	4	14 7	11.5	20 7	2	1	3
8	3	15 0	6.3	14.4	3	1	2
9	2	16 6	15 6	15 0	3	2	1
2	3	20 2	12.1	20.7	2	1	3
10	2	22.0	12.2	19 8	3	1	2
4	3	25 8	24.2	—		1	
2	2	31 7	22.5	36.4	2	1	3
6	3	32.8	21.3	37 1	2	1	3
9	1	36.3	27.9	30 5	3	1	2
10	1	36.2	21.5	32.9	3	1	
6	2	38.0	28 7	38 0	2	1	2
4	2	43.7	34 5	—	2	1	
5	3	50.9	50.1	55.[illegible]	2	1	3
5		53.5	50.1	58.7	2	1	3
8	1	56.4	52.9	63.3		1	3
2	1	62.1	50.1	57.0	3	1	2
8	2	63.3	58 7	70.2	2	1	3
4	1	69.0	55.3	—	2	1	
5	1	72.5	63.9	75 9	2	1	3
6	1	81 1	57.5	76 0	3	1	2

[a] Values listed in this column are a qualitative comparison of vessel diameters d ring the low room low oxygen periods. The number 3 represents the highest magnitude, and the number 1 represent lowest.

Table IV Sign test of differences in relative flow velocity, arteriolar diameter and volume flow

[illegible]	Mean flow velocity	Mean arteriolar diameter	Mean volume flow
at low O_2 [illegible] larger ( )	28	31	36
[illegible] O_2 [illegible] larger ( )	9	5	1
value	p 0.01	p 0.001	p 0.001
at low O_2 [illegible] larger ( )	17	25	27
[illegible] O_2 [illegible] larger ( )	13	5	4
value	p 0.30	p 0.001	p 0.001

[illegible]crease. Blood flow increased in only 1 of the 9 vessels which had a flow velocity increase and in the other 8 volume flow was virtually unchanged or decreased. When all vessels were considered together volume flow was reduced by 44 when exposed to room oxygen super[illegible] by a significant decrease in both diameter (17) and flow velocity (17) Fig. 2.

During the second low oxygen period, following exposure to room air for more than 1 h, mean volume flow returned to essentially the same level as before exposure to room air showing that the decrease in volume flow which occurred during exposure to room O_2 was not simply a progressive decrease with time. There was a tendency for diameter to increase slightly from the first low O_2 period, but volume flow remained unchanged due to a concomitant decrease in velocity.

Discussion

The exposure of living tissue for direct in vivo microscopic observation subjects it to a number of influences which may not be encountered during normal physiological conditions. The extent to which quantitative measurements of microcirculatory phenomena can be extrapolated to the normal physiological state will depend upon the similarity of the environ-

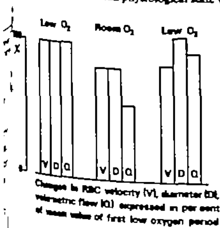


Fig 2 Changes in arteriolar diameter, red cell velocity and blood flow in percent of first low oxygen period values.

TABLE III Mean of three calculated arteriolar blood flow values during each period of low oxygen *room oxygen tension.*

Hamster	Vessel	Mean flow volumes			Relative[a] volume flow		
		Low O_2 nl sec⁻	Room O_2 nl sec^{-1}	Low O_2 nl sec^{-1}			
9	5	0.0023	0 0022	0.0025	2	1	3
4	6	0.033	0.002	—	2	1	
10	5	0 0082	0 0077	0.0105	2	1	3
9	4	0.007	0.004	0 006	3	1	2
1	3	0.019	0.017	0 023		1	3
1	4	0.015	0.007	0.019	2	1	3
4	5	0 060	0.034	—	2	1	
5	4	0.035	0 024	0 029	3	1	2
4	4	0.102	0 033	—	2	1	
10	4	0.023	0 022	0.027	2	1	3
2	4	0.063	0 051	0.103	2	1	3
6	5	0.041	0 013	0 065	2	1	3
1	2	0.080	0 039	0.069	3	1	2
9	3	0 023	0.013	0 011	3	2	1
8	4	*0.054*	*0.014*	*0 094*	2	1	3
10	3	0 117	0 123	0 099	2	3	1
1	1	0 108	0 067	0 130	2	1	3
6	4	0.199	0.068	0 219	2	1	3
8	3	0.189	0 016	0 182	3	1	2
9	2	0 170	0 134	0.11	3	2	1
2	3	0.360	0.068	0 114	3	1	2
10	2	0.322	0.14	0.253	3	1	2
4	3	1 698	0 900	—	2	1	
2	2	1.583	0 241	0 696	3	1	2
6	3	1.325	0.298	0.703	3	1	2
9	1	1 099	0.516	0 735	3	1	2
10	1	0.555	0 343	0 655	2	1	3
6	2	3 3 4	2.022	2.219	3	1	2
4	2	6.881	4.020	—		1	
5	3	2.467	2.168	2 632	2	1	3
5	2	6 421	3 474	5.294	3	1	2
8	1	5.278	4.918	4.543	3	2	1
2	1	8.367	5.236	7.879	3	1	2
8	2	3 816	2.639	3 121	3	1	2
4	1	14 607	10.613	—		1	
5	1	10 166	4 991	12.386	2	1	3
6	1	16 756	6.086	6 209	3	1	2

Values listed in this col mn re a qualitative comparison of the low room, nd low oxygen perio The number 3 represents the t me period w th the greatest magnitude while the number 1 represents t lowest magnitude.

calculated. The values obtained are listed in Table III A qualitative comparison was us for a statistical analysis by sign test and the results are presented in Table IV

When comparing the values for *diameter* measured during *the room* oxygen period wi the values obtained during the initial low oxygen periods, 31 vessels showed a decreas diameter one vessel was unchanged and 5 vessels showed an increased diameter Mean flo velocity decreased in 28 vessels and increased in 9 vessels following the shift to the roo oxygen tension period. All 5 vessels which increased in diameter as a result *of the* shift to t room oxygen tension had a decrease in flow velocity which caused total blood flow

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ment of the tissue during the experiment to that existing under normal physiological conditions.

One factor which must be considered in attempting to simulate normal physiological conditions is the oxygen tension to which a tissue is exposed Duling (1972) has demonstrated that the PO of the suffusion solution covering the hamster cheek pouch preparation has an effect upon arteriolar diameter In this study Duling demonstrated a significant decrease in the diameter of large arterioles (30–50 μm) when the suffusion solution PO was raised from 11 mmHg to 47 mmHg When the suffusion solution PO was raised from 11 mmHg to 84 mmHg there was a significant decrease in diameter only in terminal arterial vessels (12 μm). However since the blood flow through a vessel will depend not only upon the diameter of the vessel but also upon the pressure difference along the vessel, blood flow must be measured directly to predict flow changes Alternatively both pressure and diameter changes could be measured simultaneously to predict volume flow changes.

In the study presented in this paper we have therefore measured the change in the volume of blood flowing through the arteriolar vessels of the hamster cheek pouch when the tissue was exposed to a suffusion solution with an oxygen tension of <30 mmHg and then to $PO_2 > 115$ mmHg. It was found that upon elevation of the suffusion solution PO_2 to 1[illegible] mmHg, volume blood flow decreased in 36 of the 37 vessels measured. When all vessels were considered together there was a significant decrease in both arteriolar diameter and flow velocity The most consistant finding was, however a 44% decrease in blood flow O[illegible] results would therefore confirm the diameter measurements made by Duling (1972) showing a decrease in vessel diameter with increasing PO and also provide proof for his assumption that these diameter changes reflect a decrease in blood flow Although one might predict a decrease in blood flow as a result of vasoconstriction the relation between diameter, red cell velocity and blood flow is complex and one cannot predict the magnitude of the blood flow reduction unless all branching orders of vessels can be shown to constrict.

In the study by Arfors *et al* (1975 b) it was shown that an increase in oxygen tension from 5 mmHg to room oxygen tension reduced capillary blood flow to zero. Skeletal muscle as a metabolicly active tissue might be more responsive to such alterations than the connective tissue part of the hamster cheek pouch From these results and as found in our present study it can be seen that quantitative measurements of flow dependent phenomena may be greatly altered by exposing a tissue to room oxygen tension.

The experimental model presented in this paper could not distinguish between an indirect effect of oxygen through the parenchymal tissue or a direct effect upon the smooth muscle of the vasculature itself In a recent study however Duling and Pittman (1975) have shown that in the hamster cheek pouch the effect of oxygen upon vascular diameter is not direct upon arteriolar smooth muscle, but probably through its effect on the metabolism of the parenchymal tissue

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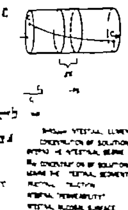


Fig. 1 Schematic illustration of the mathematical model used in the present study when analysing the ^{85}Kr absorption data

...ger partly or fully from being absorbed via the blood by diffusing from the subepithelial capillary network to the central arterial vessel so that it is brought back towards the villous tips. This hypothesis implies that the time for such intervascular diffusion in the vascular loops and, hence, the linear blood flow rate is the main determinant of the rate of krypton absorption. Evidence for this hypothesis was obtained in the studies by Svanvik and coworkers (Svanvik 1973 a). In fact, the countercurrent mechanism seemed to be the most important determinant of the rate of absorption of ^{85}Kr when blood flow was reduced by lowering the perfusion pressure (Svanvik 1973 a, b).

The studies on ^{85}Kr absorption referred to above were performed with a technique in which a saline solution containing ^{85}Kr was perfused at a high rate (30–80 ml/min) through the intestinal lumen, in an attempt to reduce intraluminal concentration gradients. This experimental situation can hardly be called "physiological" and the present study was therefore undertaken to investigate the relationship between blood flow and rate of krypton absorption when intraluminal perfusion was slower (1 ml/min). Furthermore, the effect of varying the intraluminal pressure on absorption rate was studied.

Methods

Theoretical considerations. The method utilized in this study is based on a mathematical framework originally developed by Renkin (1959) for transcapillary solute exchange. Fig. 1 illustrates a schematic ... the model. By dividing the intestinal lumen into a great number of consecutive segments (one of them, ... shown in the Fig.) applying Fick's law to each segment and integrating over the entire intestinal segment, the following solution is obtained (Renkin 1959)

$$E = 1 - e^{-PS/Q} \tag{1}$$

E denotes the fractional extraction for the solute, P intestinal "permeability", S intestinal mucosal surface and Q flow through the intestinal lumen (see Fig. 1).

The fractional extraction, E, can also be estimated when knowing the concentration of the solution entering (C_i) and leaving (C_o) the intestinal segment.

$$E = \frac{C_i - C_o}{C_i} = 1 - e^{-PS/Q} \tag{2}$$

$$PS = -Q \ln(1 - E)$$

Acta physiol. scand. 1977 100 412–423

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The Importance of the Intestinal Countercurrent Exchanger fo ⁸⁵Kr Absorption from the Feline Gut

By

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Received 28 January 1977

Abstract

JODAL, M J SVANVIK and O LUNDGREN *The importance of the intestinal counten exchanger for ⁸⁵Kr absorption from the feline gut* Acta physiol. scand. 1977 100 41

The rate of ⁸⁵K absorption from the feline gut was studied at varying intestinal blood flows induce infusions of a vasodilator drug o by lowering arterial inflow pressure. The effects on rate of ab of distendi g the intestine by increasing ntraluminal pressure from 0–1 to 5–7 cm H_2O as ell as menting the rate of l minal perfusion of the ⁸⁵Kr solution were also investigated. Distending t bowel increased rate f ⁸⁵Kr absorptio at all levels of intestinal blood flow except at the very low very high blood flow rates. Decreasing blood flow by lowering of arterial perfusion pressure d the rate of bsorption from the distended gut while it had no effect in the collapsed small intes creasing the rate of luminal perfusion enhanced the rate of ⁸⁵Kr bsorption at all blood flow leve t the lowest ones. The results are discussed with regard to villous hemodynamics, I testinal counte exchange and intraluminal concentration gradients i the lumen. It is concluded that the counte exchanger represents, under physiological conditions, the major limiting factor for the bsorption phlic solutes such as ⁸⁵Kr

The intestinal epithelium is a relatively impermeable structure for water soluble solu to the rather low permeability of the epithelial plasma membranes for such substanc because of the "tightness" of the intercellular junctions. As regards lipid soluble comp intestinal permeability is considerably higher because of the lipid nature of the cell branes. These permeability differences are also reflected in the relationship betw testinal blood flow and the rate of absorption for the different solutes. Thus, it ha shown that absorption of water soluble solutes are not flow-dependent (Winne 19 all probability due to the epithelium acting as a rate limiting barrier for the absorpti

The rate of absorption of lipid soluble compounds has been found to vary with t of blood flow and this observation has been interpreted as evidence for a blood flow l absorption of lipid soluble solutes (see *e.g.* Winne 1971 Levitt and Levitt 1973). Ho in recent reports from this laboratory the intestinal countercurrent exchanger was fo play a significant role in the absorption of lipid soluble solutes (Biber *et al.* 1973 b, S 1973 a, b). According to the countercurrent hypothesis krypton is "hindered" by t



Fig. 3. The correlation between total intestinal blood flow and ^{85}Kr intestinal absorption rate at high (5–7 cm H_2O) and low (0–1 cm H_2O) intraluminal pressures. Vasodilatations were produced by graded close i.a. infusions of isopropylnoradrenaline. The lumen of the gut was perfused at a rate of 1 ml/min. Mean ± S.E.

Autoradiographic technique. To study the distribution scheme in the intestinal wall of the absorbed tracer, an autoradiographic study was performed using the lipid soluble N-methyl-^{14}C-antipyrine (New England Nuclear Chemicals). ^{85}Kr was not suitable for this part of the study because of its comparatively low energy β radiation and the difficulties inherent in avoiding its evaporation from thin tissue slices. The autoradiographic technique was identical to that used earlier in this laboratory (Kampp *et al.* 1968). In the experiments saline solution containing N-methyl-^{14}C-antipyrine (35–55 μCi/100 ml) was perfused through an isolated intestinal segment for 30–40 min, as described above, after which the segment was immediately extirpated and frozen in isopentane precooled by liquid nitrogen. In half of the experiments the intestinal lumen was, prior to extirpation, rapidly flushed with 2–3 ml saline. The frozen segment was cut in a cryostat (system Dittes Duisburg) at −20°C into 10–20 μm thick transverse slices. The section was mounted on glass and heated to 65–70°C for about 30 s. They were then placed in close contact with dental x-ray film (Kodak), and exposed at room temperature together with a drying agent (silica gel), for varying periods of time, the time depending on the amount of radioactivity in the tissue. The localization of the tracer in the intestinal wall, as indicated by the blackness of the autoradiographs, was determined by densitometer recording and by simultaneous microscopical examination of the histological section and its corresponding autoradiograph.

Control experiments to rule out any marked diffusion artefact with this autoradiographic technique have been reported earlier (Kampp *et al.* 1968).

Statistics. Statistical significance was calculated by Wilcoxon's non-parametric tests (Siegel 1956). A P value of 0.05 or less was judged as significant.

Results

The absorption rate of ^{85}Kr was studied at two different levels of intraluminal pressure (P_L), i.e. at about 0–1 cm H_2O (below named low P_L) and at 5–7 cm H_2O (below named high P_L). At low P_L the intestinal lumen was largely collapsed while increasing intraluminal pressure to 5–7 cm H_2O, a pressure well within the physiological range (Hamson 1973, Öhman 1974), created a cylindrical intestine without appreciably distending the intestinal wall or affecting the intestinal blood flow.

The measured extractions (E, see Methods), from which rate of ^{85}Kr absorption was calculated, usually ranged between 0.15 and 0.30, rarely exceeding 0.50. In no experiment did E reach 1.0.

Increasing P_L induced a statistically significant increase of the rate of ^{85}Kr absorption at all blood flow levels studied, except at maximal vasodilatation when total intestinal blood flow exceeded 200 ml/(min × 100 g) (see Fig. 3). The absorption rate at high P_L had reached

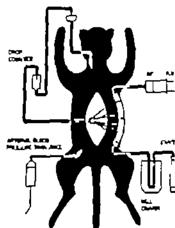


Fig. 2. The experimental technique used in this investigation for studying rate of ^{85}Kr absorption schematically illustrated.

The PS product, expressed in *e.g.* ml/min 100 cm² serosal surface, corresponds to the clearance of from the intestinal lumen. This product is dependent on the overall permeability of the intestinal … the mucosal surface area available for absorption and on the luminal perfusion rate but also on intralum… concentration gradients ("unstirred layers") and on the rate of blood flow. The PS-product will therefor… this context largely depend on factors other than those originally described for capillaries by Renkin (1… and the word "permeability-surface" was hence in this study considered misleading. The term … absorption rate" reflects more correctly the meaning of the PS-value in this study and is therefore u… below.

B. Operati… and experimental procedures. The experiments were performed on cats anesthetized [… chloralose (50 mg/kg b.wt.) after induction with ether. The animals were deprived of food for 24 h and … no obvious signs of intestinal infection. After tracheotomy the abdomen was opened in the midline and the … greater omentum and spleen were extirpated. The experiments were performed on a 15–20 cm long iso… segment from the proximal jejunum, the remainder of the gastrointestinal tract being extirpated. … lumen of the segments was carefully rinsed with bodywarm saline.

After heparinizing the animal (3–5 mg/kg b.wt.) blood pressure was measured by pressure transduc… (Statham P 23 AC) in the femoral artery and in the experiments where intestinal hypotension was induc… also in a branch to the superior mesenteric artery. The superior mesenteric vein, draining the intestinal … segment and its lymph nodes, was cannulated and connected to an optical drop recorder—ordinate … unit, recording on a Grass polygraph. The venous blood was returned to the animal via the right jugu… vein, the venous outflow pressure being kept around 7 mmHg.

Both splanchnic nerves were cut while the parasympathetic supply to the small intestine was left inta… Vasodilatation was induced by constant infusions of isoprenaline, 5–10 µg/min, through a side branch … the superior mesenteric artery. Reduction of the arterial inflow pressure was achieved by an adjustal… clamp placed around the superior mesenteric artery.

^{85}Kr was either dissolved in isotonic saline or in half of the experiments, in an isotonic mannitol solutio… no difference in rate of ^{85}Kr absorption was seen between the two solutions. The ^{85}Kr solution was perfu… through the intestinal segment (Fig. 2) at a rate of 1 ml/min from a 100 ml syringe driven by an infusi… pump. The radioactivity in the perfusate leaving the intestinal segment was continuously recorded … directing the perfusion solution through a well type scintillation detector operating a spectrometer (Packa… Auto-Gamma Spectrometer series 410 A) coupled to a ratemeter and the Grass polygraph. The coun… measured by the ratemeter were intermittently estimated by manual digital counting. The perfusion rate w… measured distal to the well counter by an optical drop recorder unit, the outlet of which could be adjust… so as to give any desired intraluminal pressure. It was set at 0–1 cm or 5–7 cm above the level of the dist… end of the intestinal segment. The perfusion system consisted of glass in order to avoid any loss of radio… activity by diffusion into the tubing or the environment. The ^{85}Kr absorption rate was measured duri… steady state conditions, *i.e.* after at least 20–25 min of constant blood flow and constant rate of perfusi… entering and leaving the intestinal segment. Furthermore, the counting rate had to remain constant for a… least five min. At the end of each experiment the radioactivity in the syringe solution was counted by intro… ducing it into the wellcounter via the same perfusion system as used for the perfusate.

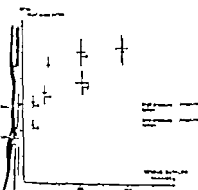


Fig. 3. The correlation between total intestinal blood flow and ^{85}Kr intestinal absorption rate at high (5–7 cm H_2O) and low (0–1 cm H_2O) intraluminal pressures. Vasodilatations were produced by graded close i.a. infusions of isopropylnoradrenaline. The lumen of the gut was perfused at a rate of 1 ml/min. Mean ± S.E.

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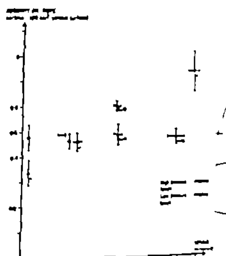


Fig. 4 The relationship between intestinal blood flow and rate of ⁸⁵Kr absorption when decreasing rate of blood flow by reducing arterial blood pressure in the superior mesentery artery. Zero blood flow was accomplished by clamping simultaneously the intestinal artery and vein. The lumen of the gut was perfused at a rate of 1 ml/min. Mean ± S E.

its maximum at a blood flow level somewhat above 100 ml/(min 100 g), while at low P_L vascular bed had to be intensely vasodilated before the same absorption rate was attained.

Fig. 4 illustrates the effect on the rate of ⁸⁵Kr absorption of a decreased blood flow achieved by a graded reduction of the arterial inflow pressure down to a pressure head about 30 mm Hg. This experimental procedure had no appreciable effect on the rate absorption recorded at low P_L, while the rate recorded at high P_L was reduced in proportion to blood flow. The statistically significant difference between low and high P_L observed "resting" blood flows (*cf* Fig. 3), was not observed at the lowest blood flow levels (Fig. A further reduction of the blood flow down to zero, produced by clamping the blood vessels supplying the intestinal segment, caused no further reduction in ⁸⁵Kr absorption rate high P_L, but a decrease at low P_L.

The influence of the luminal perfusion rate, and hence of increased stirring of the luminal contents, on the absorption rate of Kr is illustrated in Fig. 5 and 6. The data from the higher perfusion rates, expressed per 100 g intestinal tissue were obtained with the technique described by Biber *et al* (1973 b) where the intestinal lumen was perfused in a closed perfusion system. The actual intraluminal pressure was not measured in these experiments

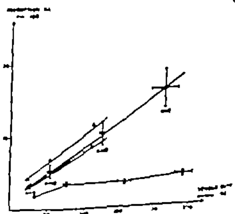


Fig. 5 The rate of ⁸⁵Kr absorption plotted versus total venous outflow from the small bowel at varying luminal perfusion rates. The values illustrated for 80 and 30 ml/min are identical to those reported by Biber *et al.* (1973 b). The observations made at a perfusion rate of 50 ml/min represent previously unpublished results. The results for 1 ml/min are identical to those obtained at a high P_L, illustrated in Fig 3. The lines indicating the blood flow-absorption rate relationship were drawn according to the method of least squares at the three high perfusion rates. Mean ± S E.

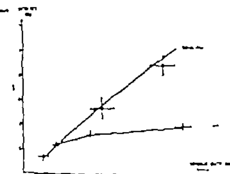


Fig. 6. The relationship between rate of ^{85}Kr absorption and total intestinal blood flow at two different rates of luminal perfusion. Blood flow was decreased from the resting control level by lowering arterial perfusion pressure by partially occluding the superior mesenteric artery. The results illustrated from 1 ml/min are those recorded at a high luminal pressure (*cf.* Fig. 4). The line indicating the relationship observed at a perfusion rate of 50 ml/min was constructed according to the method of least squares. Mean ± S.E.

It seems reasonable to assume that P_L was above 0·1 cm H_2O since the gut was cylindrical. At "resting" blood flow, perfusion of the intestinal lumen with 30, 50 or 80 ml/min increased the ^{85}Kr absorption rate 3 times compared to that measured in the present study at high P_L. This difference was drastically augmented during vasodilatation (Fig. 5) and the ^{85}Kr absorption rate then increased linearly with increased blood flow up to 3 ml/(min · 100 g) during the high luminal perfusion rates, while this was not the case at the luminal perfusion rate of 1 ml/min.

When blood flow was decreased by lowering arterial inflow pressure, a marked reduction of the rate of ^{85}Kr absorption was observed during high rates of luminal perfusion. In this experimental situation ^{85}Kr absorption remained significantly higher than at the low rate of luminal perfusion, until blood flow was reduced to 20–25 per cent of control (Fig. 6). At this low blood flow level, the pressure head then being only about 30 mm Hg, the absorption rate at the luminal perfusion of 1 ml/min was similar to that observed at a luminal perfusion of 50 ml/min. Thus, at this low blood flow rate, the absorption rate of ^{85}Kr was independent both of intraluminal pressure (Fig. 4) and of the rate of luminal perfusion (Fig. 6).

The autoradiographic study with N-methyl-^{14}C-antipyrine demonstrated that in all experiments every villi were blackened on the autoradiographs *i.e.* they were all exposed to the solution in the intestinal lumen. Furthermore, only the tip portion of the villi seemed to be in efficient contact with the intestinal contents to judge from the pronounced grain density at the tip and the densitometric gradient observed along the length of the villi (Fig. 7).

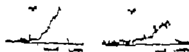


Fig. 7. Densitometric tracings of autoradiographs indicating the distribution of N-methyl-^{14}C-antipyrine in the intestinal wall after a 20 min period of luminal perfusion of the tracer in a saline solution. The experiment of panel A was performed at a low P_L while the experiment illustrated in panel B was performed on the same cat at a high P_L. Thin horizontal lines indicate densitometric baselines. Exposure time for autoradiographs 19 days.

The histological sections also showed that upon increasing P_L the villi tended to spr making the intervillous space larger

Discussion

The present study has demonstrated that the absorption rate of ^{85}Kr an inert lipoph solute, in some way is related to the magnitude of intestinal blood flow However t flow-absorption relationship differs considerably depending on both the intraluminal pr sure (P_L) and on the luminal perfusion rate in the intestinal segment. During low lumi perfusion (1 ml/min) and high P_L (5–7 cm H O) the rate of ^{85}Kr absorption increased wh blood flow was raised from 5 to about 100 ml/(min 100 g). Above that blood flow level t rate of ^{85}Kr absorption seemed to level off (Fig. 3 and 4). At a low P_L (0-1 cm H_2O) th absorption rate remained more or less constant in the lower range of blood flows (5-30 (min 100 g)), but increased continuously between 30 and 250 ml/(min 100 g) (Fig. 3 a 4) At the two extreme values of blood flow (5 and 250 ml/(min 100 g)) the rate of sorption at high and low P_L coincided. At all other levels of blood flow the rate of absorption was greater at high than at low P_L. At high rates of luminal perfusion (arou 50 ml/min) an almost linear relationship between total intestinal blood flow and absorpti rate was observed At all levels of blood flow except the very lowest (5–10 ml/(min × 100 g) ^{85}Kr absorption rate was higher at a high luminal perfusion rate than at a low one. Hen at a low blood flow rate, absorption rate of ^{85}Kr was independent of P_L or luminal pe fusion rate. It should in this context be underlined that the direct observations of this stu made it possible to relate absorption rate to *total* blood flow However changes in to blood flow may not necessarily reflect qualitatively or quantitatively similar changes i villous blood flow (*cf* Fig. 8)

Fig. 8 and 9 summarize some background information of importance for the followi discussion Fig. 8 illustrates how villous blood flow and mean transit time in the villo vascular loops are related to total intestinal blood flow These data were originally publish in reports by Biber *et al* (1973 a) and by Lundgren and Svanvik (1973) In Fig. 9 the tim required for a certain diffusion equilibrium between the outer surfaces and the midpoi of different cylinders are given assuming a diffusion constant of 10^{-5} cm²/s for the free diffusing solute. Comparing these two figures it is evident that a considerable fraction of t easily diffusable solute, such as ^{85}Kr may be trapped in the intestinal countercurrent e changer of the cat, having an intervascular distance of about 20 μm.

Villous blood flow and ^{85}Kr absorption rate The vascular architecture of the villus in a num ber of different animals including the cat suggests that *all* the blood supplying the villo tissue passes the villous tip (for references, see Lundgren 1967). Furthermore, this is prob ably the only part of the villus that is in "effective" contact with the luminal contents at "physiological" luminal pressure (*cf* Fig. 7 Hamilton *et al* 1967). This suggests that t mucosal surface area is of no critical importance for the rate of absorption of easily di fusible solutes. This conclusion is further corroborated by calculating, from Fig. 9 the tim required for a 95 per cent diffusion equilibrium across the intestinal epithelium (heigh

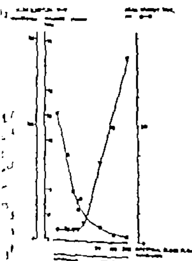


Fig. 8 Schematic summary of the hemodynamics in the feline villi. The figure illustrates the changes in villous blood flow and villous mean transit time when increasing intestinal blood flow by i.a. infusions of isopropylnoradrenaline ("vasodilatation") or decreasing blood flow by reducing arterial inflow pressure to the gut. A detailed account of the illustrated observations can be found in Biber et al. (1973) and Lundgren and Svanvik (1973). Note that the scale on the abscissa is logarithmic.

[illegible] 20 μm) and translating this value into capillary length using the data of Fig. 8 (assumed villous length 0.7 mm). Such a computation reveals that the assumed diffusion equilibrium is reached along a capillary length of 20–30 μm at "rest" and of about 120 μm during intense vasodilatation.

The latter calculation strongly suggests that the passage of ⁸⁵Kr across the intestinal epithelium does not represent a limiting step in the ⁸⁵Kr absorption. The calculation even suggests that the absorption of ⁸⁵Kr may be totally blood flow limited, also when the intestinal vascular bed is maximally dilated and the absorption of ⁸⁵Kr is restricted to the tip portion of the villus. At least two observations of this study are, however not consistent with this conclusion. Firstly comparing absolute values (Fig. 5 and 6 with Fig. 8) it is evident that the rate of ⁸⁵Kr absorption was at all levels of blood flow always below that of villous blood flow. Secondly the changes in rate of absorption of krypton, induced by altering blood

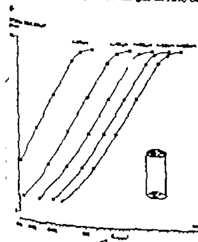


Fig. 9 The theoretically calculated time required for reaching certain diffusion equilibrium between the outer surface of a cylinder and its center at varying radii of the cylinder (r). The diffusion was assumed to occur freely in water, the diffusion constant being 10^{-5} cm²/s. The calculations are based on Figure 217 in Davson (1970).

flow were similar to the changes in villous blood flow at a luminal perfusion rate of 1 ml/m and a low P_L (compare Fig. 3 4 and 8), suggesting a flow limitation for gas absorpt However then the rate of absorption could always be increased by distending the gut. T observation cannot be accounted for in terms of an increased absorptive surface area (above) or in terms of a blood flow limitation Hence, other factors besides rate of bl flow must also be involved in determining the rate of ^{85}Kr absorption.

Intraluminal concentration gradients and ^{85}Kr absorption rate It was a striking finding in the present study that increasing the rate of luminal perfusion markedly augmented the r of ^{85}Kr absorption (Fig. 5 and 6) although it never reached a rate corresponding to the r of villous blood flow This may possibly be explained by the stirring effect of the intraluminal perfusion (*cf* Biber *et al* 1973 b), minimizing *e g* the creation of intralum concentration gradients of the tracer However it was surprising to find that the rate of absorption from a collapsed intestine (luminal perfusion at 1 ml/min) with a low intralum pressure usually was below that observed at a high luminal pressure, since the creation intraluminal gradients would *a priori* seem more probable in the distended gut. Moreov the luminal volume was smaller in the collapsed situation than in the distended one, wh the rate of perfusion was identical in the two situations (1 ml/min) Hence, the "stirrin effect of the perfusion must have been more pronounced at a low P_L, suggesting that t presence of intraluminal concentration gradients, and/or unstirred layers (*cf* Dietsc 1973) was of no crucial importance in the present experiments, at least at a low P_L At hi P_L and augmented total intestinal blood flow the shape of the blood flow-absorption cur (Fig. 3) suggests the presence of a diffusion limitation probably secondary to intralumi concentration gradients. The recorded effect on ^{85}Kr absorption of an increased rate luminal perfusion is probably mainly explained by gas absorption also occurring in oth parts of the mucosa than the villi (see Biber *et al.* 1973 b)

Countercurrent exchange and Kr absorption rate Distension of the intestine causes t villi to spread, increasing their "functional absorptive area. However the latter factor probably of no importance for the rate of absorption of easily diffusible lipophilic solut as discussed above On the other hand, the spreading of the villi affects the efficiency of t intestinal countercurrent exchanger since in the collapsed state only the very tip of t villus may be in good functional" contact with the intestinal contents, while in the distend situation the intervillous spaces are more easily accessible (*cf* Fig. 7) Hence, in the co lapsed state the exchanger is long" and highly efficient in hindering net blood absorptio of lipophilic solutes. In the distended gut, on the other hand, the "effective" length of th exchanger is decreased, shortening the time available for cross-diffusion in the exchange This, in turn, reduces the efficiency of the exchanger and may augment the rate of ^{85}K absorption

From the discussion above it is clear that the increase of intraluminal pressure from 0- to 5–7 cm H_2O alters at least three parameters of importance for passive intestinal ab sorption of lipophilic solutes, *i.e* distending the gut may increase absorptive surface area increase diffusion distances and decrease the efficiency of the countercurrent exchanger It has been pointed out above that changes in intestinal surface area or in concentratio gradients cannot explain the present findings. It is proposed that the effect of distension o

[illegible] absorption rate is explained by alterations in the efficiency of the countercurrent ex[illegible] [illegible]. Hence, the exchanger seems to be one of the major limiting factors for passi[ve] [illegible] absorption of lipophilic solutes, such as ^{85}Kr These conclusions are based on the [fol]lowing observations made in this study

1. At high luminal perfusion rates the rate of krypton absorption decreased when lowering [lumi]nal perfusion pressure although villous blood flow was unchanged (compare Fig. 4 [and] [illegible]). This is explained by the countercurrent hypothesis as a result of the increased mean [tra]nsit time in the villi that is induced at low perfusion pressure, augmenting the efficiency [of] the exchanger in "hindering" net blood transport of the tracer (Fig. 8).

2. At very low intestinal blood flow rates (around 5 ml/min 100 g) the rate of ^{85}Kr [abso]rption was independent of both luminal perfusion rate and P_L, although the absorption [rate] was only 1/4–1/3 what would have been expected, had it been blood flow limited. Ac[cord]ing to the countercurrent hypothesis, the rate of ^{85}Kr absorption was in this situation [alm]ost completely hindered by the exchanger as regards its net blood transport. In other [wor]ds, at the long mean transit times in the villi (20–30 s) the net blood transport of the [trac]er was completely hindered and the slow rate of absorption was essentially determined [by] the diffusion of the tracer along the villus axis to the deeper mucosal layers where no [exch]anger existed. This situation largely persisted at somewhat higher rates of blood flow [in] the collapsed intestine (Fig. 4) due to the higher efficiency of the exchanger in this situa[tio]n (see above).

3. The rate of ^{85}Kr absorption at zero blood flow by diffusion from lumen to serosa was [of] the same order of magnitude as that observed at low blood flows (Fig. 4). This observa[tion] strongly suggests that at low blood flows the absorption of ^{85}Kr was almost entirely [dep]endent on diffusion along the villus axis (*cf* point 2 above). The relatively low absorp[tio]n rate observed in the collapsed intestine in the *in vitro* situation may be explained by the [fact] that the diffusion distance for ^{85}Kr in that situation (lumen to serosa) was longer than [in] the *in vivo* situation (lumen to blood vessels in deeper parts of mucosa and/or submucosa). [Th]e difference between the distended and the collapsed gut, persisting also at no blood [circ]ulation, was expected since the diffusion distance between lumen and serosa was prob[ab]ly comparatively short when the villi are spread out at high P_L, allowing the intestinal [lu]minal to reach more deeply between villi.

Combining the results of this study with the measurement of villous blood flow obtained [in] this laboratory by Svanvik and coworkers (Biber *et al.* 1973 a, Lundgren and Svanvik [197]3), it is possible to obtain a rough estimation of the efficiency of the countercurrent [ex]changer for krypton when varying villous blood flow Such calculation has been per[for]med in Table I which is based on the measurements performed in this study at low P [Th]e data were used assuming that no concentration gradients for ^{85}Kr existed in the in[test]inal lumen, *i* the rate of absorption was considered not to be limited by the rate of [diff]usion in the lumen. The rate of absorption at intestinal blood flows below 35 ml/min 100 [g] 0.4 ml/min 100 g) was, in accordance with the discussion above, presumed to be solely [du]e to a diffusion in the villi along the gradient established by the exchanger (Fig. 7), which [fur]thermore was assumed to be essentially similar at all rates of villous blood flow Sub[trac]ting this diffusive absorption it was possible to obtain a measure of the rate of blood

TABLE I The calculated diffusive absorption and "blood flow absorption of 85Kr at varying bl flow levels in the feline gut.

Blood flow ml/min 100 g intestinal tissue		Rate of absorption ml/min × 100 g intestinal tissue			Blood flow absorption in of villous blood flow
Total intestinal flow	*Villous flow*	*Observed*	*By diffusion*	*By blood flow*	
10–35	4.5–14	1 40	1 40	0	0
65	32	2.05	1 40	0.82	26
160	55	2.65	1 40	1.59	[illegible]9
45	75	4 00	1 40	3.28	44

Corrected for a water blood partition coefficient of 1.26 (Lassen and Munck 1955).

flow absorption" Since the solubility for 85Kr is higher in water (saline) than in blood values for blood flow absorption in Table I have been corrected for by the water bl partition coefficient for 85Kr reported by Lassen and Munck (1955). Table I dea demonstrates that the rate of 85Kr absorption to the blood stream is at all levels of blood fl only a *small* fraction of what one would expect, had 85Kr absorption been purely blood fl limited. This high efficiency of the exchanger in hindering net blood flow transport v *a priori* expected (*cf* Fig. 8 and 9 *cf* the kidney Aukland 1967).

The discussion above clearly suggests that the intestinal countercurrent exchanger pl an important role in the absorption of lipid soluble inert gases in the feline gut, as was a pointed out in earlier investigations by Biber *et al* (1973 b) and by Svanvik (1973). T significance of the intestinal exchanger for the absorption of fatty acids and sodium v studied by Jodal (1973) As regards sodium the exchanger acts as a countercurrent multipl producing a very high tissue osmolality at the villous tip in much the same way as in t kidney (Jodal and Lundgren 1975).

Evidence for the presence of an intestinal countercurrent exchanger in the dog was recen provided by Bond and Levitt (1976) However in three other reports from the same resear group (Levitt and Levitt 1973 Bond *et al* 1974 Micflikier *et al* 1976) they claimed to ha obtained no experimental support for any intestinal countercurrent exchanger using th rats or rabbits. In the paper by Levitt and Levitt (1973) the absorption data obtained on t rat from the stomach, small bowel and colon of five inert gases were fitted into five differe physico-mathematical models. One of the models seemed to predict the absorption ra from all the consecutive segments of gastrointestinal tract, although there are no reaso to assume that one single model would be valid for the perfusion–diffusion interaction in gastrointestinal segments with its large variation in vascular anatomy The proposed mod implied that the gas absorption from the small intestine was almost entirely blood fl limited However the results could as well be interpreted in accordance with the counte current hypothesis, a conclusion the authors considered as unlikely (Micflikier *et al* 197 The disparate findings of Levitt *et al* were instead explained in terms of species di ferences. It was pointed out that the rabbit villi are shorter and leafshaped and that they a furthermore, less densely packed which may allow for intestinal contents to be in goo contact also with the base of the villi However any experimental support for this view w not presented. The presence of a hyperosmolar region in the villous tip in the rabbit vi

bold and Lundgren, unpublished observations) strongly indicates the presence of an changer also in the intestinal villi of this animal.

Note After submitting this report for publication, a study on the dog by Bond *et al* (*J. Clin. Invest* 77 :9 306-310) was published, which largely confirms the conclusions of this investigation.

This research was supported by grants from the Swedish Medical Research Council (14X 2855), from Svenska Läkaresällskapets Fund, from Harald and Greta Jeansson Fund, From Wilhelm and Martina Lundgrens Fund, from Magnus Bergvalls stiftelse and from the Faculty of Medicine University of Göteborg

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Acta physiol scand. 1977 100. 424-429
From the Institute of Physiology University of Lund, Sweden

Evidence of Decarboxylation of Lysine by Mammalian Ornithine Decarboxylase

By

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Received 10 February 1977

Abstract

PERSSON L. *Evidence of decarboxylation of lysine by mammalian ornithine decarboxyl*
Acta physiol. scand. 1977 *100* 424-429

In enzymic preparations from mouse kidney stimulated with the anabolic steroid Durabolin (nandrol phenpropionate) lysine and ornithine were shown t inhibit the decarboxylation of each other competit The Michaelis constants for the decarboxylations were approximately equal to the inhibition constan the two amino acids. The pH optima of the decarboxylation of lysine and ornithine ere found t identical. Chromatographic studies of the enzyme preparation on a Sephadex G-150 Superfine col did not bring about separation f the two enzyme activities. The ratio of the decarboxylating activ was practically the same during the elution. Lysine decarboxylating activity was also shown to be pr in growth hormone stimulated rat liver The results are in greement with the assumption that the carboxylation of lysine and ornith ne is carried ut by the same enzyme.

The biosynthesis of cadaverine (1 5-diaminopentane) has been closely studied in mi organisms but only a few investigations are available concerning vertebral animals developing chick embryos Caldarera, Barbiroli and Moruzzi (1965) found a parallel incr of the contents of cadaverine and putrescine (1 4-diaminobutane) reaching maximum va at five days of incubation Cadaverine from a source independent of bacterial decarbox tion of lysine in the intestine, has been shown to be present in the brain of mice (Ste Klauco and Dolezalova 1974) Recently this laboratory reported that administration of anabolic steroid Durabolin (nandrolone phenpropionate) to mice gave rise to an elev content of cadaverine in the kidneys and the appearance of an enzyme catalyzing the de boxylation of lysine (Henningsson, Persson and Rosengren 1976) Furthermore the act of ornithine decarboxylase in the kidneys of mice was greatly enhanced after Durab administration (Henningsson, to be published)

Lysine and ornithine are homologous it is hence possible that the decarboxylation of two amino acids is carried out by the same enzyme. The problem has been studied in present investigation.

Table 1 Effect of growth hormone on decarboxylation of lysine and ornithine in rat liver. Lysine decarboxylating activity (LDC) was determined in incubation mixtures containing supernatant corresponding to 340 mg of tissue, 0.20 μmol pyridoxal-5-phosphate, 20 μmol ^{14}C-lysine (10 μCi mmol) and sodium phosphate buffer in a final volume of 3.0 ml. Incubation time was 60 min. Incubation mixtures, where ornithine decarboxylase activity (ODC) was measured, consisted of supernatant corresponding to 100 mg of tissue, 0.20 μmol pyridoxal-5-phosphate, 0.20 μmol ^{14}C-ornithine (40 μCi mmol) and sodium phosphate buffer in a final volume of 2.0 ml. Incubation time was 30 min. Enzymic activities are expressed as nmol CO_2 formed per g rat liver and hour. Mean and the S.E. of mean are given, n = 5

	ODC	LDC
Controls	0.45 ± 0.239	94 0.713
Growth hormone	164.03 ± 9.778	43.49 ± 2.658

Methods

Conventional male NMRI mice (30–40 g b. wt.) and female rats of Sprague–Dawley strain (165–180 g b.wt.) were used in this study. The animals were fed a standard diet ad libitum.

Treatment of animals

The mice received 0.1 mg Durabolin (Organon), nandrolone phenpropionate, suspended in arachis oil, daily for 3 days subcutaneously. The kidneys were removed 18–20 h after the last injection.

Four hours before sacrifice 0.1 U of growth hormone (Somacton, Ferring, Malmö) per 100 g b.wt. were administered to the rats intraperitoneally in its original solvent. The control animals received solvent only. The liver was used for enzyme assay.

Preparation of tissue extracts

The animals were killed by cervical dislocation and exsanguinated. The appropriate organs were rapidly removed and dissected free of fat and capsule (kidney). The tissue samples were first minced with scissors and then gently homogenized in a Dounce type homogenizer (25 strokes with the pestle) in 7 (kidney) or 4 (liver) volumes of cold 0.1 M sodium phosphate buffer containing 10^{-4} M EDTA, 5 × 10^{-4} M dithiothreitol and 0.2 % (w/v) glucose (referred to sodium phosphate buffer in the following text). The pH of the buffer was 7.2 except when otherwise stated. The homogenate was centrifuged at 20 000 g for 30 min at 4 C. The supernatant was, except when used for determining influence of pH on the decarboxylating enzyme, dialysed at 4 C overnight against 2 × 2.5 l of sodium phosphate buffer.

Analytical methods

The decarboxylation of ornithine and, in some experiments, the decarboxylation of lysine was measured by determining the release of $^{14}CO_2$ from DL-[1-^{14}C]-ornithine and DL-[1-^{14}C]-lysine respectively. The composition of the incubation mixtures and the incubation times are shown in the legends to the Table and Figures. The incubations were done at 37 C in vessels equipped with a 10 × 25 mm piece of no. 005 Munktell filter paper prepared with 100 μl of hydroxide of Hyamine 10-X. The reactions were stopped by addition of 0.5 or 0.7 ml 2 M perchloric acid from a side arm of the vessel. To obtain complete absorption of released $^{14}CO_2$ into the hydroxide of Hyamine 10-X, the incubation vessels were shaken for additional 45 min. The filter papers were then placed in vials containing 8 ml of scintillation mixture (Bray 1960) and the radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer.

Decarboxylation of lysine was in some experiments estimated by measuring the decarboxylation product cadaverine. The incubation mixture consisted of supernatant corresponding to 5.0 mg of mouse kidney, 10^{-4} M pyridoxal-5-phosphate, ^{14}C L-lysine of various concentrations, 10^{-4} M aminoguanidine sulphate (to inhibit any action of diamine oxidase) and sodium phosphate buffer in a final volume of 2.0 ml. ^{12}C-ornithine (10^{-4} M) was added when studying the inhibition of the decarboxylating enzyme. After 30 min incubation at 37°C the reaction was terminated by adding 0.7 ml sulfosalicylic acid (final conc. 4 %). The cadaverine content of the incubate was measured by using an automatic amino acid analyzer LKB-BIOCAL 201, with a column of Durrum DC 6 A. Application of this technique for amine determination has been described by Hatanaka (1970).

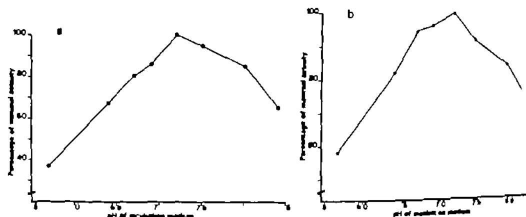


Fig. 1 The influence of pH on lysine decarboxylating activity (a) and ornithine decarboxylating act. (b). In (a) the incubate contained supernatant corresponding to 100 mg of mouse kidney 0.010 µ pyridoxal-5-phosphate, 0.10 µmol ^{14}C lysine (0.5 mCi/mmol) and sodium phosphate buffer of various in a final volume of 1.0 ml. Incubation time was 60 min. Mean of duplicate incubations, except for activity at pH 8.0 which is the mean of triplicate incubations. In (b) supernatant corresponding to 15 of mouse kidney was incubated for 30 min with 0.010 µmol pyridoxal-5-phosphate, 0.10 µmol ^{14}C-ornit (50 µCi/mmol) and sodium phosphate buffer of various pH in a final volume of 1.0 ml. Mean of duplic incubations.

Purification of the enzyme

Dialyzed supernatant fluid was fractionated with $(NH_4)_2SO_4$ at 3 C. Supernatant corresponding to 3- of mouse kidney was used. Solid ammonium sulphate (70 mg/ml) was added to obtain 10% saturation. The mixture was stirred for 20 min and centrifuged at 25 000 g for 10 min. The precipitate was discarded. To remaining supernatant additional 280 mg/ml of solid $(NH_4)_2SO_4$ was added. This gave 50% saturation of the salt. Stirring and centrifugation resulted in a pellet with most of the ornithine and lysine decarboxylating activity. The pellet was dissolved in 5 ml of the same phosphate buffer as used for homogenization, dialysed at 1°C overnight against 2.5 l of the same medium. The solution was then applied to Sepha G-150 Superfine column (90 × 2.5 cm) equilibrated with sodium phosphate buffer at 1°C. The prot were eluted at a flow rate of 2.5–3.0 ml/h and fractions of 5–6 ml were collected for enzyme assay.

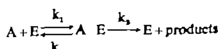
Results and Discussion

Enzymic activity as related to pH

The lysine decarboxylating activity and the ornithine decarboxylating activity were maxi at pH 7.2 (Fig. 1). The fact that two reactions have the same pH optimum should no taken as evidence of a single enzyme catalyzing these reactions. On the other hand, if amino acids, as chemically similar as lysine and ornithine, are decarboxylated by the s enzyme it is most likely that the pH optima of the decarboxylating activities are identic

Enzyme kinetics

For an enzyme E acting on a substrate A the reaction scheme can be written as



$$A + E \underset{k}{\overset{k_1}{\rightleftharpoons}} A\ E \xrightarrow{k_3} E + \text{products}$$

According to Lineweaver and Burk (1934) the reaction rate v is given by the equation

$$1/v = (K/V)(1/a) + 1/V \qquad K = \frac{k_2 + k_3}{k_1}$$

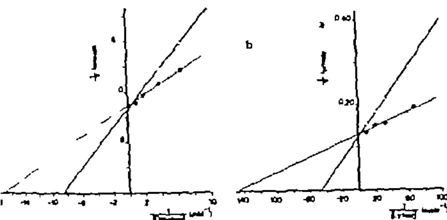
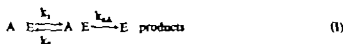


Fig. 2. Effect of substrate concentration on ornithine decarboxylating activity (a) and lysine decarboxylating activity (b). In (a) supernatant corresponding to 2.5 mg of mouse kidney was incubated for 30 min with various concentrations of ^{14}C-ornithine (50 μCi/mmol), 0.20 μmol pyridoxal-5-phosphate and sodium phosphate buffer in a total volume of 2.0 ml (●). ^{12}C-L-lysine (10^{-3} M) was added when inhibition was studied ( ). Each point is the average of three assays. In (b) the reaction rate was determined by measuring the decarboxylation product, i.e. cadaverine. Analysis of enzymic activity was made in the presence (○) or in the absence (●) of ^{12}C-L-ornithine (10^{-3} M). Incubation conditions are given in Methods. Each point is the average of three assays.

V represents the maximal reaction rate and a the concentration of substrate A. Plotting $1/v$ versus $1/a$ gives a straight line with an intercept of $-1/K$ on the $1/a$ axis.

If the enzyme acts on two different substrates (A and B) the scheme of reaction will be



$$A + E \underset{k_{2A}}{\overset{k_{1A}}{\rightleftharpoons}} A\,E \xrightarrow{k_{3A}} E + \text{products} \qquad \text{(I)}$$



$$B + E \underset{k_{2B}}{\overset{k_{1B}}{\rightleftharpoons}} B\,E \xrightarrow{k_{3B}} E + \text{products}_B \qquad \text{(II)}$$

If the two substrates are present at the same time there will be a competition for the active sites on the enzymes. Thus B will behave as a competitive inhibitor of reaction (I) and A as a competitive inhibitor of reaction (II).

The rate of breakdown of A can be derived from the equation (for derivation see Reiner 1959 p. 169)

$$1/v_a = 1/a(K_a/V_a)(1 + b/K_b) + 1/V_a \qquad (2)$$

where

$$K_a = \frac{k_{2A} + k_{3A}}{k_{1A}} \qquad K_b = \frac{k_{2B} + k_{3B}}{k_{1B}}$$

v_a stands for the rate of breakdown of A, V_a for the corresponding maximal rate, a for the concentration of A, b for the concentration of B.

For reaction (II) there is a similar equation

$$1/v_b = 1/b(K_b/V_b)(1 + a/K_a) + 1/V_b \qquad (3)$$

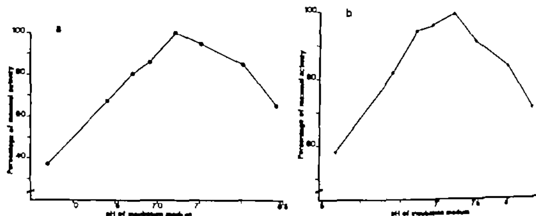


Fig. 1 The influence of pH on lysine decarboxylating activity (a) and ornithine decarboxylating activity (b) In (a) the incubate contained supernatant corresponding to 100 mg of mouse kidney 0 010 μmol pyridoxal-5-phosphate, 0.10 μmol [14]C-lysine (0.5 mCi/mmol) and sodium phosphate buffer of various pH in a final volume of 1 0 ml. Incubation time was 60 min. Mean of duplicate incubations, except for the activity at pH 8 0 which is the mean of triplicate incubations. I (b) supernatant corresponding to 3 mg of mouse kidney was incubated for 30 min with 0 010 μ mol pyridoxal-5-phosphate, 0.10 μmol [14]C-ornithine (50 μCi/mmol) and sodium phosphat buffer of various pH in a final volume of 1.0 ml. Mean of duplicate incubations.

Purification of the enzyme

Dialysed supernatant fluid was fractionated with $(NH_4)_2SO_4$ at 3°C. S pernatant corresponding to 3-4 g of mouse kidney was used. Solid ammonium sulphate (70 mg/ml) was added to obtain 10 saturation. The mixture was stirred for 20 min and centrifuged at 25 000 g for 10 min. The precipitate was discarded. To the remainl g supernatant add tional 280 mg/ml f solid $(NH_4)_2SO_4$ was added. This gave 50 saturation of the salt. Stirring and centrifugation resulted in a pellet with most of the ornithine and lysine decarboxylating activity The pellet was dissolved n 5 ml of the same phosphate buffer as used for homogenization and dialysed at 1 C overnight against 2.5 l of the same medium The solution was then applied to a Sephadex G 150 Superfine column (90 2.5 cm) equilibrated with sodium phosphate buffer at 1°C. The proteins were eluted t flow rat of 2.5–3 0 ml/h and fractions of 5–6 ml were collected for enzyme assay

Results and Discussion

Enzymic activity as related to pH

The lysine decarboxylating activity and the ornithine decarboxylating activity were maximal at pH 7.2 (Fig 1) The fact that two reactions have the same pH optimum should not be taken as evidence of a single enzyme catalyzing these reactions. On the other hand, if two amino acids, as chemically similar as lysine and ornithine, are decarboxylated by the same enzyme it is most likely that the pH optima of the decarboxylating activities are identical.

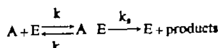
Enzyme kinetics

For an enzyme E acting on a substrate A the reaction scheme can be written as

$$A + E \underset{k}{\overset{k}{\rightleftharpoons}} A\ E \xrightarrow{k_s} E + \text{products}$$

According to Lineweaver and Burk (1934) the reaction rate v is given by the equation

$$1/v = (K/V)(1/a) + 1/V \quad K = \frac{k_s + k_s}{k} \qquad (1$$



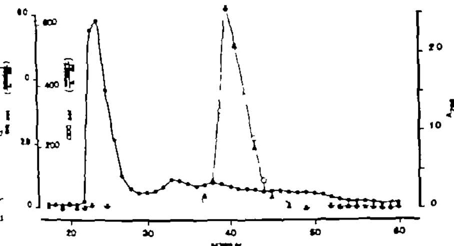


Fig 3 Sephadex G-150 Superfine chromatography of enzyme preparation. Assay mixtures contained 100 µl (LDC) or 20 µl (ODC) eluent, 0.10 µmol pyridoxal-5-phosphate, sodium phosphate buffer and 0.36 µmol ^{14}C-lysine (1 mCi/mmol) or ^{14}C-ornithine (50 µCi/mmol) in final volume of 1.0 ml. Incubation time was 30 min. Enzymic activities are given in nmol per ml eluent and hour. LDC = lysine decarboxylating activity (O), ODC = ornithine decarboxylating activity ( ), A280 = absorbance at 280 nm (●).

apparently high value of decarboxylation of lysine in control liver was not searched for but was most likely due to a nonenzymatic release of ^{14}CO

This work was supported by grant from the Swedish Medical Research Council B77-04X-02212 10C.

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If b is constant and a is varied plotting 1/v versus 1/a gives a straight line with the intercept of $-(1/K_a)/(1+b/K_b)$ on the 1/a axis. The effect of the presence of B is thus to produce an apparent increase of K_a by the factor $(1+b/K_b)$. If b is known it is possible to determine K_b from the magnitude of the apparent increase of K_a. In a similar way K_a can be estimated by keeping a constant and varying b.

Consequently if ornithine and lysine were decarboxylated by the same enzyme the presence of lysine should competitively inhibit the decarboxylation of ornithine and vice versa. As seen in Fig. 2 lysine and ornithine inhibited the decarboxylation of one another in a competitive manner (Lineweaver and Burk 1934). Measurements of the decarboxylation of ornithine gave in accordance with formula (1) a $K_{ornithine}$ of 6×10^{-4} (Fig. 2 a). K_{lysine} was similarly determined to 0.7×10^{-4} (Fig. 2 b). By using incubation mixtures containing both amino acids, where the concentration of one of them was held constant and the concentration of the other was varied, the magnitudes of the apparent increases of $K_{ornithine}$ and K_{lysine} were estimated according to formula (2) and (3) (Fig. 2). From these magnitudes $K_{ornithine}$ was calculated to 5×10^{-4} and K_{lysine} to $1.1 \times 10^{-}$. Thus, K_{lysine} and $K_{ornithine}$ obtained from indirect determinations were in good agreement with the values determined directly. This finding supports the view that lysine and ornithine were decarboxylated by the same enzyme. Another circumstance favouring this assumption was that putrescine inhibited both the ornithine decarboxylating activity and the lysine decarboxylating activity to a higher degree than did cadaverine (data not shown).

Chromatographic study of the enzyme

Supernatant obtained from mouse kidney stimulated with Durabolin was fractionated with ammonium sulphate as described in Methods. The preparation containing most of the lysine and ornithine decarboxylating activities was applied to a column of Sephadex G-150 Superfine and the chromatographic distributions of the enzyme activities were examined. The elution patterns given in Fig. 3 shows that separation of the two activities was not achieved on gel filtration. Furthermore the ratio of the decarboxylating activities throughout the peak was practically constant. Thus these chromatographic studies support the view that decarboxylation of lysine and ornithine was carried out by the same enzyme.

Enzymic activity in rat liver

The results related to above indicated a decarboxylation of lysine by ornithine decarboxylase. Hence the question arose whether an enzyme capable of decarboxylating lysine existed in tissues other than mouse kidney stimulated with Durabolin.

Growth hormone administration to rats has been shown to enhance liver ornithine decarboxylase activity (Jänne, Raina and Siimes 1968; Jänne and Raina 1969). It would thus appear that if lysine and ornithine are decarboxylated by the same enzyme lysine decarboxylating activity should also be present. As seen in Table I growth hormone treatment, besides an increase of ornithine decarboxylase, resulted in an induction of lysine decarboxylating activity in rat liver. Furthermore the ratio of the two enzyme activities in growth hormone stimulated liver was equal to that found in mouse kidney. The cause of the

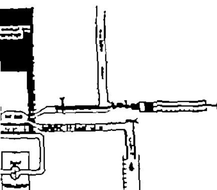


Fig. 1. Experimental set-up. For explanation, see text.

sion for these indicators across the blood to tissue exchange barriers of adipose tissue. er anatomical possibilities will then have to be considered as candidates for transendothelial pathways for hydrophilic molecules.

rimental procedure

riments were performed on female rabbits of the Danish country breed. After 12 h of fasting thesia was induced with sodium pentobarbitone 25 mg/kg. Supplementary doses were given as ired. Rectal temperature was kept constant at 38°C. Tracheostomy was made and an endotracheal was introduced. Blood pressures from right atrium and carotid artery were measured via polyethylene tubing leading to Statham strain-gauge transducers connected to a Varian writer and an oscilloscope. inguinal fat pad was isolated as described earlier (Nielsen 1972, Paaske and Nielsen 1976). All blood els leading to and from the pad were ligated except for the superficial epigastric artery and vein . 1). The pad was wrapped in polyethylene sheets and placed in a lead shield kept at 37°C by water ulation. The femoral artery was ligated 1.5 cm distal to the superficial epigastric artery. The blunt end the femoral artery was placed in the longitudinal direction of the superficial epigastric artery. After ation of the animal the femoral vein was catheterized distal to the branching of the superficial gastric vein so as to allow collection of effluent venous blood from the pad. Thus, the effluent blood was collected and to compensate for loss of blood, donor whole blood was given through the catheter the right atrium. 150 μg (30 μg/ml) unlabelled cyanocobalamin was injected through this catheter into bloodstream. After 5 min the blunt end of the femoral artery was cannulated with the needle (external meter 0.4 mm) of a microsyringe. The tip of the needle was led as close as possible to the fat pad in the men of the superficial epigastric artery. A silk ligature was made to compress the artery to the needle. (500 μCi) ^{58}Co-cyanocobalamin (specific activity 150–300 μCi/μg cyanocobalamin. The Radiochemical tre Ltd., Amersham, Great Britain) was injected, the ligature was removed and the needle was withdrawn from the area. The whole injection procedure with stopped flow had a total duration of 2–2.5 s. tivity in the field was recorded by a one inch NaI (Tl) scintillation detector (PW 4111 Type A, Philips, Netherlands) which was collimated to see the fat pad only. The scintillation detector was connected to single-channel pulse gamma spectrometer (Meditronik a/s, Denmark) adjusted around the 0.122 MeV peak of ^{58}Co. Activity was printed out as count rate for each s. Via an interface unit the spectrometer was connected to a tape puncher (Type 4070, AB Facit, Sweden) providing a paper tape used for computer . Activity was recorded for 300 s. Effluent blood from the pad was collected, and volumes per time unit of haematocrit value were determined. Blood flow was stopped 300 s after injection. Counts remaining in field after removal of the pad were measured and used as a background which was subtracted from the recorded count values.

Calculations

The curve function was analysed on a WANG 600 process computer in accordance with the principles described earlier (Paaske and Nielsen 1976). The count value of the injected

Acta physiol. scand 1977 100 430–436

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Absence of Restricted Diffusion in Adipose Tissue Capillaries

By

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Received 21 February 1977

Abstract

PAASKE, W P *Absence of restricted diffusion in adipose tissue capillaries.* Acta physiol. scand 1977 *100* 430–436.

Capillary permeability in adipose tissue for ^{57}Co-cyanocobalamin (^{57}Co-B12) was determined by the single injection, external registration method. The capillary diffusion capacity CDC, (the permeability-surface area product, PS) was 1 1 ml/100 g min at a capillary extraction of 0.21 and a plasma flow of 6.7 ml/100 g min. Results were compared to ^{51}Cr EDTA data from a previous study with similar method and preparation. As CDC(^{51}Cr EDTA)/CDC(^{57}Co-B12) was 1.81 and as D(^{51}Cr EDTA)/D(^{57}Co-B12), the ratio between the free diffusion coefficients in water at 37°C, was 1 79 it is concluded that restricted diffusion does not occur in cutaneous tissue for Co-B12 as compared to ^{51}Cr EDTA, *i.e.* ^{51}Cr EDTA and ^{57}Co-B12 diffuse across the capillary membrane of adipose tissue at rates proportional to their respective free diffusion coefficients in water The Pappenheimer equivalent pore radius estimate of 30 Å and the Karnovsky interendothelial 40 Å slit width are both defective in explaining the experimental data. The transendothelial channel system of fused vesicles (Simionescu, Simionescu and Palade 1975) is a possible structural equivalent for the present findings. The results support the hypothesis that capillaries of continuous type exhibit similar permeation characteristics regardless of the tissue in which they are located.

The present study was undertaken in order to determine the capillary diffusion capacity CDC of Co-cyanocobalamin (Co-B12) in adipose tissue. In a previous study (Paaske and Nielsen 1976) CDC for ^{51}Cr-ethylene-diamine-tetraacetate (Cr EDTA) was determined applying the single injection, external registration method (Sejrsen 1970) to the rabbit inguinal fat pad preparation. As identical experimental procedures are used the CDC values should be comparable to allow examination of whether the ratio between the CDC values deviates from the ratio between the free diffusion coefficients in water *i.e.* whether restricted diffusion is present in adipose tissue for ^{57}Co-B12 as related to ^{51}Cr EDTA.

A physiological implication of presence of restricted diffusion for ^{57}Co-B12 as compared to ^{51}Cr EDTA is that the Pappenheimer equivalent pore radius estimate of 30 Å (Pappenheimer Renkin and Borrero 1951 Perl 1971) can be considered applicable to adipose tissue capillaries. Consequently the Karnovsky (1967 1968) interendothelial slit might be a possible morphological substrate of the pore. However if restricted diffusion is absent for Co-B12 as compared to ^{51}Cr EDTA in adipose tissue as in cutaneous and muscular tissues (Paaske 1977 a and b) it must be assumed that the pore is so wide as to allow free

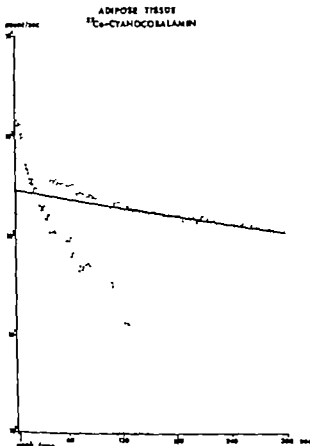


Fig. 2. Experimental curve (expt. No. 1). The dots denote the recorded count rates corrected for background activity. The monoexponential regression line was calculated in the interval from 150 to 300 s after the bolus injection of ⁵⁷Co-cyanocobalamin. Crosses signify the intravascular transit curve obtained by subtraction of the regression line from the original count values (dots). For details, see text.

An experimental curve (expt. No. 1) is shown in Fig. 2. Recorded activity corrected for background values (dots) was plotted by the computer as a function of time in a semilogarithmic diagram. At time zero 5 µl (500 µCi) ^{57}Co-B12 was given as a bolus. The maximum count value of 1 722 cps was reached at the 2nd s. As computed in the time interval from 150 s to 300 s after the injection the slope of the regression line was −0.00324 s^{-1} with a standard deviation of 0.00018 s, giving a value of 283 at peak time. The capillary extraction was (283/1722) = 0.164. The mean transit time of tracer in plasma was 0.346 min leading to a plasma flow of f(pl) = (1/0.346) (1 − 0.305) 0.03 100 (ml/100 g min) = 6.0 ml/100 g min. Consequently CDC = −6.0 0.94 ln (1 − 0.164) (ml/100 g min) = 1.0 ml/100 g min.

Discussion

In an earlier study (Paaske and Nielsen 1976) the method and its application to the fat pad preparation was discussed in detail. Also, it was demonstrated that the capillary diffusion

TABLE I Capillary extraction, E, and capillary diffusion capacity CDC, of [a] Co-cyanocobalamin in adipo tissue. P_d is the permeability coefficient as calculated from an assumed capillary surface are of 35 cm /g. $\bar{x}$ is mean value and S.E. is standard error of the mean.

Exp. No	E per cent	CDC ml/100 g min	P_d 10^6 cm/s	Plasma flow ml/100 g min		Weight of fat pad g	Hct fraction	Peak time s
				Direct	Calculated			
1	16.4	1.0	0.48	7.0	6.0	17 7	0.31	
2	14.3	1.3	0.62	7.8	8.8	15.1	0 31	9
3	12.9	0.9	0.43	9 8	7.3	8.2	0 37	2
4	18.2	1.0	0 48	7 4	5 1	5.9	0 31	3
5	15 4	1 4	0 67	6.5	8.6	7 1	0 34	2
6	46.1	1 0	0.48	1.8	1.8	14.2	0 38	11
$\bar{x}$	20.6	1 1	0.53	6.7	6.3	11 4	0.34	5
S.E.	5.2	0.1	0.04	1 1	1 1	2.0	0 01	2

[a] Co-B12 was taken as the highest recorded count value. Peak time was the time at whic this occurred The part of the curve recorded from 150 s to 300 s after injection was mono exponentially extrapolated to peak time using "least square" regression analysis. This fu was subtracted from the original curve as to provide the intravascular transit functio Intravascular mean transit time was calculated from the Zierler (1965) equation t(iv) are height Plasma flow was calculated as f(pl) = (1/t(iv)) λ 100 (ml/100 ml min) (Kety 195 where λ is intravascular plasma volume. λ was estimated at 0 03(1 − Hct) (ml/g) whe Hct is hematocrit value (Paaske and Nielsen 1976) Capillary extraction, E, was given the value of the monoexponentially extrapolated curve at peak time expressed as a fracti of the maximum count value. The capillary diffusion capacity CDC defined as the un directional flux of indicator across the capillary membrane per 100 g tissue per un concentration difference across the capillary (Lassen and Trap-Jensen 1968), was calculat as CDC = − f(pl) K ln (1 − E) (ml/100 g min) where K is a constant for converting n plasma to ml interstitial fluid. A K value of 0 94 was employed (Lassen and Trap-Jens 1968)

Results

The experimental results are presented in Table I Mean value of capillary extraction [a] Co-B12 in adipose tissue was 0.21 with a standard error of the mean, S E., of 0 032 in expts. The mean capillary diffusion capacity CDC, was 1 1 ml/100 g min (S.E. 0.1 m 100 g min) The permeability coefficient P_d was calculated from the assumption th capillary surface area in adipose tissue is about half the value reported from skele muscle. S was estimated to 35 cm /g (Gersh and Still 1945) using the skeletal muscle data Pappenheimer Renkin and Borrero (1951). Consequently P_d was calculated to 0.53 10 cm/s (S.E. 0 04 10^{-6} cm/s) The directly measured plasma flow was 6.7 ml/100 g m (S E. 1 1 ml/100 g min) and the plasma flow was 6.3 ml/100 g min (S E. 1 1 ml/100 g mi as calculated from the externally recorded curve function by kinetic analysis. The me weight of the fat pad was 11 4 g (S E. 2.0 g) and mean hematocrit value was 0.34 (S.E 0 01) Mean peak time was 5 s (S.E. 2 s)

Traditionally blood to tissue transport of hydrophilic substances has been believed to take place through the Pappenheimer equivalent pore system which includes a circular cylindrical waterfilled pore of 30 Å radius or alternatively a slit width of 37 Å. As a structural equivalent for the Pappenheimer pore, Karnovsky (1967, 1968) suggested the interendothelial slit. The results of the present study are not compatible with the Pappenheimer and Karnovsky models as transcapillary exchanges of ^{51}Cr-EDTA and ^{57}Co-B12 take place with rates proportional to their respective free diffusion coefficients. However the Simionescu, Simionescu and Palade (1975) channels of fused vesicles might prove to be the morphological equivalent to the physiological findings and it is tempting to assume that this system is the pathway for hydrophilic substances across the wall of the continuous capillary (Palade 1977 a).

It is concluded that restricted diffusion does not exist in adipose tissue for ^{57}Co-B12 as compared to ^{51}Cr-EDTA. The Pappenheimer equivalent pore radius estimate of 30 Å or the slit width of 37 Å and the Karnovsky slit are defective in explaining the present data. The transendothelial system of fused vesicles (Simionescu, Simionescu and Palade 1975) might prove to be the structural pathway for the observed transcapillary exchange. The hypothesis is supported that capillaries of continuous type exhibit similar permeation characteristics regardless of the tissue in which they are located.

Dr Per Sejrsen is gratefully acknowledged for advice throughout the study and for criticism of the manuscript. Supported by the Danish Medical Research Council.

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capacity of ⁵¹Cr EDTA CDC(⁵¹Cr EDTA) was 2.0 ml/100 g min at a capillary extraction of 0 264 and a plasma flow of 7 4 ml/100 g min. The ratio CDC(⁵¹Cr-EDTA)/CDC(⁵⁷Co-B12) can now be calculated as 2.0 (ml/100 g min)/1 1 (ml/100 g min) 1 82. The ratio between the free diffusion coefficients in water at 37°C for the two indicators is D(⁵¹Cr EDTA)/D(⁵⁷Co-B12) = 0 700 10^{-5} (cm²/s)/0.390 10^{-5} (cm²/s) = 1 79 (Paaske 1977 a). As 1 82 ≈ 1 79 it is concluded that capillaries of adipose tissue do not exhibit restricted diffusion to ⁵⁷Co-B12 as compared to ⁵¹Cr EDTA.

Although the literature provides no information directly comparable to the present series, some data of Linde Chisolm and Rosell (1974) will be considered Using the indicator diffusion method of Crone (1963) the CDC of ¹⁴C-sucrose (molecular weight, MW 342.3) and ³H polyethyleneglycol (³H PEG MW 800–1 000) were determined in canine adipose tissue CDC(¹⁴C-sucrose) was about 2 ml/100 g min and CDC(³H PEG) was about 1 ml/100 g min giving a CDC(¹⁴C-sucrose)/CDC(³H PEG) ratio of about 2. The authors do not provide information concerning the free diffusion coefficient for the ³H PEG fabrication used in their study Using the formula $a = (3MW/4\pi gN)^{1/3}$ where g is specific gravity and N is the Avogadro constant the Stokes-Einstein molecular radius of the equivalent sphere, $a_{s\ x}$(³H PEG), can be estimated at 6.2–6 7 Å This corresponds to a free diffusion coefficient in water at 37°C of 0 49–0.53 10^{-5} cm²/s Using the relation $D\sqrt{MW}$ = constant, estimates of D(³H PEG) give 0 42–0 46 10^{-5} cm²/s. As D(¹⁴C sucrose) is 0 686 10^{-5} cm²/s, the ratio D(¹⁴C-sucrose)/D(³H PEG) can be estimated to be 1.3–1 6. As the CDC ratio 2 is larger than 1 3–1 6 the data of Linde, Chisolm and Rosell (1974) would indicate some degree of restricted diffusion to ³H PEG as compared to ¹⁴C sucrose. As the estimates mentioned above of D(³H PEG) are subject to considerable uncertainties and as no experimental determination has been performed for this substance conclusive statement is impeded on whether or not the ³H PEG ¹⁴C-sucrose data are in concert with the findings of the present study

The capillary wall of adipose tissue consists of a monolayer of endothelial cells which is surrounded by a continuous basement membrane Endothelial cells contain mitochondria endoplasmic reticulum and numerous vesicles and vacuoles. As in muscular tissue (Simionescu, Simionescu and Palade 1975) vesicles and vacuoles can be seen to form transendothelial channels (Blanchette-Mackie and Scow 1971 Scow *et al* 1976). Tight junctions with slits are located between endothelial cells. On these morphological grounds the capillaries were called "continuous" capillaries, and their structure resembles closely that seen in muscle, myocardium, lung, and cutaneous tissue (Bennet, Luft and Hampton 1959 Majno 1965). The present findings could therefore be comparable to data obtained in other tissues with capillaries of continuous type In cutaneous and muscular tissue restricted diffusion was not present for ⁵⁷Co-B12 as compared to ⁵¹Cr EDTA (Paaske 1977 a and b) With other indicators and methods it has been demonstrated that also myocardium (Alvarez and Yudilevich 1969 Bassingthwaighte Yipintsoi and Grabowski 1975) and lung (Boyd *et al* 1969 Normand *et al* 1971) exhibit permeation characteristics that can be considered similar to those of adipose tissue These permeability studies with extracellular hydrophilic indicators have led to the hypothesis that the permeability characteristics of the continuous capillary appear to be the same regardless of the tissue in which it is located (Paaske 1976).

Acta physiol. scand. 1977. 100. 437–445
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Transcapillary Exchange of ^{14}C Inulin by Free Diffusion in Channels of Fused Vesicles

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Received 21 February 1977

Abstract

PAASKE, W. P. and P. SEJRSEN, *Transcapillary exchange of ^{14}C-inulin by free diffusion in channels of fused vesicles*. Acta physiol. scand. 1977. *100*. 437–445.

Capillary permeability for the extracellular, hydrophilic indicator ^{14}C-inulin, was determined in the isolated cat gastrocnemius muscle by bolus injection, venous sampling expt. The capillary diffusion capacity CDC (the permeability-surface area product, PS), was 0.84 ml/100 g min, and capillary extraction, E, = 0.307 at plasma flow of 4.0 ml/100 g min as average values of 7 expts. Results were compared with ^{51}Cr-EDTA and ^{57}Co-B12 data of previous studies. The findings imply that ^{14}C-inulin is not subject to restricted diffusion across the continuous capillary membranes of skeletal muscle as compared to ^{51}Cr-EDTA as well as ^{57}Co-B12. These hydrophilic indicators pass the capillary barrier at rates proportional to their respective free diffusion coefficients in water. The Pappenheimer equivalent pore radius estimate of 30 Å (or slit width of 37 Å) and the Karnovsky 40 Å interendothelial slit width are inconsistent with the present data which imply a much larger pore size. The transendothelial channel system of fused vesicles is a possible morphological equivalent for the present findings which support the general theory that capillaries of continuous type exhibit similar permeation characteristics regardless of the tissue in which they are located. By linear black-box analysis the extravascular distribution volume for ^{14}C-inulin in skeletal muscle was found to be 13.0 ml/100 g. The data indicate that ^{51}Cr-EDTA, which has about the same molecular radius as sucrose, and ^{14}C-inulin have identical extravascular volumes of distribution and that both molecules presumably enter the sarcoplasmic reticulum.

Studies on diffusional exchange of hydrophilic solutes across the barriers of continuous capillaries in skeletal muscle and in adipose and cutaneous tissues have demonstrated that ^{51}Cr-ethylene-diamino-tetraacetate (^{51}Cr EDTA, corrected molecular radius, a_e, 5.1 Å) and ^{57}Co-cyanocobalamin (^{57}Co-B12, a_e 8.9 Å) pass the endothelium at rate constants proportional to their respective free diffusion coefficients in water at 37°C (Paaske 1977 a, b, c). The Pappenheimer, Renkin and Borrero (1951) concept of a 30 Å equivalent pore radius and the Karnovsky (1967, 1968) 40 Å interendothelial slit width are both inconsistent with these results as a larger pore size would be necessary to account for the experimental findings of absence of restricted diffusion. The present study aims at determining the capillary diffusion capacity CDC (the permeability—surface area product, PS), in skeletal muscle for a larger molecule, viz. ^{14}C-inulin, which has a corrected molecular radius, a_e, of 15.3 Å. This procedure allows determination of whether the ratio CDC(^{51}Cr-EDTA)/CDC(^{14}C-inulin) indicates restricted diffusion as found by Pappenheimer or whether ^{14}C-inulin is not subject

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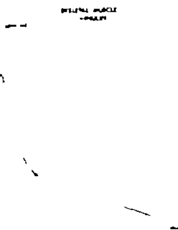


Fig. 1 The initial 15 min of the curve obtained in expt. No. 7. C(t) is plotted as function of time in semilogarithmic diagram. The extravascular transit was isolated by extrapolation (punctured line) of the regression line (solid line). For procedure of calculations, see text.

The part of the curve as indicated in Table III (Extrapolation interval ev) was monoexponentially extrapolated to time zero using the least square method (Fig. 1 representing expt. No. 7) in analogy with extrapolation procedures used in previous studies (Sejrsen 1979, Paaske and Nielsen 1976). The back extrapolated part of the curve followed by the registered curve and the extrapolation to infinity of the final slope was taken to represent extravascular transit of indicator. Area under this extravascular curve divided by area under total curve gave capillary extraction, E.

$$E = \left[\int_0^{\infty} c(t)\,dt\right]_{ev} \Big/ \left[\int_0^{\infty} c(t)\,dt\right]_{total} = A(ev)/A(total) \qquad (2)$$

The numerical values of the extravascular curve function were subtracted from values of the registered curve to give the intravascular transit curve. Intravascular mean transit time,

Table II. Experimental results. $\bar{t}$ denotes mean transit time of indicator and V is volume of distribution. iv = intravascular plasma, ev = extravascular, sr = sarcoplasmic reticulum. For explanation, see text.

Expt. No.	$\bar{t}$ (total) min	$\bar{t}$ (iv) min	$\bar{t}$ (ev) min	$\bar{t}$ (sr) min	V (total) ml/100 g	V (iv) ml/100 g	V (ev) ml/100 g	V (sr) ml/100 g	k (final) min⁻¹
1	11.06	2.50	17.51	51.81	9.95	1.82	8.13	4.95	0.0193
2	12.08	1.33	22.00	41.84	13.86	2.21	11.65	9.66	−0.0239
3	8.27	1.66	16.81	39.68	10.75	2.02	8.73	5.89	−0.0252
4	3.22	0.42	20.16	34.80	15.13	2.63	12.50	11.86	−0.0289
5	4.47	0.64	17.55	44.84	21.90	2.42	19.48	13.32	−0.0223
6	3.35	0.38	27.43	44.25	23.45	2.68	20.77	18.86	−0.0226
7	1.98	0.29	10.30	27.55	12.15	2.10	10.05	5.05	0.0363
	6.35	1.03	18.82	40.65	15.31	2.27	13.04	9.94	−0.0255
11	1.02	0.31	1.99	2.95	2.02	0.1	1.92	1.95	0.0021
A	16.43	6.00	29.86	36.23	4.93	1.90	3.03	3.20	−0.0276
B	16.64	3.21	25.36	41.15	8.32	1.91	6.41	5.67	−0.0243

TABLE I. Directly measured plasma flow capillary diffusion capacity CDC, and capillary extraction, E of ¹⁴C inulin in the autoperfused gastrocnemius muscle. P_d is the permeability coefficient calculated from CDC and an assumed capillary surface area of 70 cm²/g.

Expt No	Plasma flow ml/100 g min	CDC ml/100 g min	E fraction	P_d 10^6 cm/s
1	0.9	0.71	0.570	0.17
2	1 1	0 82	0.546	0.19
3	1 3	0 70	0 436	0.17
4	4 7	0.68	0.142	0 16
5	4.9	1 19	0.227	0.28
6	7 0	0.77	0.110	0.18
7	8.1	0 98	0 121	0 23
x	4.0	0 84	0 307	0 20
S.E.	1 1	0.07	0 077	0 02
A	0.3	0 16	0 437	0 04
B	0.5	0 44	0.606	0 10

to restricted diffusion at all which would give a CDC-ratio similar to the ratio between the free diffusion coefficients in water as found by Crone (1963 b) in expts. based on indicator diffusion By applying an indicator diffusion method alternative to that developed by Crone (1963 a) it becomes possible to determine both permeability data and extravascular volume of distribution.

Experimental procedure

Expts. were performed o 9 cats of either sex weighing 2.9 kg (mean (range 2.5 to 3 1 kg) After induction of anaesthesia an autoperfused gastrocnemius muscle preparation was made using the technique described earlier (Paaske 1977 c). A 5–7 μl bolus containing 1 10 μCi inulin-[¹⁴C]carboxylic acid (specific activity 13 mCi/mmol the R diochemical Centre, Amersham, Great Britain) was injected nto the bloodstream of the femoral artery from a side branch. For about 115 min effluent venous blood from the muscle was sampled with ppropriat time intervals. Volume per time and hematocrit value were determined throughout After termination of the expt the muscle and the contralateral gastrocnemius muscle as control removed and weighed. During the expt. dono whole blood was administered to compensat for the blood loss due to the non-recirculating system. The venous blood samples containing ¹⁴C nulin were prepared for liquid scintillation counting by centrif gation at 6 000 rpm f 10 min, collection of plasma, and preparation of plasma/water/Insta-Gel (Packard Inc.) vials. ¹⁴C-activity was recorded by Type LS-250 (Beckman Instruments Inc., Fullerton, Calif) liquid scintillation detector until either a st ndard deviation on the cpm of 0 3 per cent had been reached or the sample had been counted for 50 min.

Calculations

After correction for background activity the recorded C-inulin activities were expressed cpm/ml plasma and plotted as a function of time in a semilogarithmic diagram. Total area under the venous outflow curve from time zero to infinity A(total), was calculated from the equation

$$A(\text{total}) = \int^{\infty} c(t)\,dt = \left[\sum(\text{cpm/ml})\,\Delta t\right] + (\text{cpm/ml})\ /k(\text{final}) \qquad ($$

where τ is end time of observation, Δt is time interval represented by an individual sample and k(final) is rate constant of the final slope of the curve in min as calculated from the "least square" method (Lassen and Sejrsen 1971) in the interval indicated in Table I (Extrapolation interval – final).

Fig. 1. The initial 15 min of the curve obtained in expt. No. 7. Conc. plotted as function of time in semilogarithmic diagram. The extravascular transit was isolated by extrapolation (punctured line) of the regression line (solid line). For procedure of calculations, see text.

The part of the curve as indicated in Table III (Extrapolation interval ev) was monoexponentially extrapolated to time zero using the "least square" method (Fig. 1 representing expt. No. 7) in analogy with extrapolation procedures used in previous studies (Sejrsen 1970, Paaske and Nielsen 1976). The back extrapolated part of the curve followed by the registered curve and the extrapolation to infinity of the final slope was taken to represent extravascular transit of indicator. Area under this extravascular curve divided by area under total curve gave capillary extraction, E

$$E = \left[\int^{\infty} c(t)\,dt\right]_{ev} \Big/ \left[\int^{\infty} c(t)\,dt\right]_{total} = A(ev)/A(total) \qquad (2)$$

The numerical values of the extravascular curve function were subtracted from values of the registered curve to give the intravascular transit curve. Intravascular mean transit time,

Table II. Experimental results. $\bar{t}$ denotes mean transit time of indicator and V is volume of distribution. rv intravascular plasma, ev extravascular, sr sarcoplasmic reticulum. For explanation, see text.

Expt. No.	$\bar{t}$ (total) min	$\bar{t}$ (rv) min	$\bar{t}$ (ev) min	$\bar{t}$ (sr) min	V (total) ml/100 g	V (rv) ml/100 g	V (ev) ml/100 g	V (sr) ml/100 g	k (final) min^{-1}
1	11.06	2.90	17.51	51.81	9.95	1.82	8.13	4.95	0.0193
2	12.60	1.33	22.00	41.84	13.86	2.21	11.65	9.66	−0.0239
3	8.27	1.66	16.81	39.60	10.75	2.02	8.73	5.89	0.0252
4	3.22	0.42	20.16	34.60	15.13	2.63	12.50	11.86	−0.0289
5	4.47	0.64	17.55	44.84	21.90	2.42	19.48	13.32	0.0223
6	3.33	0.38	27.43	44.23	23.45	2.68	20.77	18.86	0.0226
7	1.50	0.29	10.30	27.53	12.15	2.10	10.05	5.05	0.0343
	6.35	1.60	18.82	40.65	15.31	2.27	13.04	9.94	0.0255
1E	1.62	0.31	1.99	2.93	2.02	0.12	1.92	1.95	0.0021
4	16.83	6.00	29.86	36.23	4.93	1.90	3.03	3.20	0.0276
3	16.64	3.21	25.36	41.15	8.32	1.91	6.41	5.67	0.0343

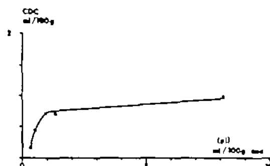


Fig. . CDC(14C-inulin) is plotted as function of plasma flow as measured from the venous outflow in a linear diagram. As calculated from the "least square" method, the function CDC(14C-inulin) = f(pl) was satisfied by the straight line equation $y = 0.73 + 0.027x$ ($r = 0.4$, $p > 0.1$) in the plasma flow interval from 0.9 to 8.1 ml/100 g min. At plasma flows 0.3 and 0.5 ml/100 g min CDC is seen to go towards zero. This is due to back-diffusion of indicator from interstitium to plasma while the bolus is still located in exchange vessels. Extraction is consequently underestimated.

$\bar{t}(iv)$, was computed as time weighted area (Aw(iv)) over area (A(iv)) of the intravascular curve from the general equation of Zierler (1965)

$$\bar{t}(iv) = \left[\int_0^{\infty} t \cdot c(t)\,dt\right]_{iv} \Big/ \left[\int_0^{\infty} c(t)\,dt\right]_{iv} = Aw(iv)/A(iv) \tag{3}$$

Plasma flow (f(pl)), as calculated from this analysis of the curve was computed from

$$f(pl) = (1/\bar{t}(iv)) \cdot \lambda \cdot 100 \ (ml/100\ g\ min) \tag{4}$$

(Kety 1951) λ denotes intravascular plasma volume which was estimated to 0.03 (1 − Hct) (ml/g) where Hct is hematocrit value (Paaske 1977 c) The capillary diffusion capacity CDC defined as the unidirectional flux of indicator across the capillary membrane per 100 g tissue per unit concentration difference across the capillary (Lassen and Trap-Jensen 1968) was calculated as

$$CDC = -f(pl) \cdot K \cdot \ln(1 - E)\,(ml/100\ g\ min) \tag{5}$$

where K is a constant for converting ml of plasma to ml of interstitial fluid. A K-value of 0.94 was used (Lassen and Trap-Jensen 1968) The permeability coefficient, P_d, was calculated from CDC/S where S is capillary surface area. The conventional estimate of S 70 cm^2/g was employed (Pappenheimer Renkin and Borrero 1951) Mean transit time of ^{14}C inulin in the whole system, $\bar{t}$(total), was calculated from time weighted area over area for the whole curve

$$\bar{t}(total) = \left[\int_0^{\infty} t \cdot c(t)\,dt\right]_{total} \Big/ \left[\int_0^{\infty} c(t)\,dt\right]_{total} = Aw(total)/A(total) \tag{6}$$

Mean transit time of indicator in the extravascular space, $\bar{t}(ev)$ was calculated from time weighted area over area for the extravascular curve

$$\bar{t}(ev) = \left[\int_0^{\infty} t \cdot c(t)\,dt\right]_{ev} \Big/ \left[\int_0^{\infty} c(t)\,dt\right]_{ev} = Aw(ev)/A(ev) \tag{7}$$

Total volume of distribution was taken as

$$V(total) = f(pl) \cdot \bar{t}(total) \tag{8}$$

[illegible] III. Variables of the kinetic curve resolution. [illegible] is end time of observation, and the extrapolation intervals denote the intervals in which "least square" regression analysis was performed.

[illegible] No.	[illegible]	Extrapol. interval	
		E[illegible] min	Final min
	120 00	10 00–30 00	45 00–115 00
	121.25	7.00–16 25	41.25–116 25
	115 00	10 00–[illegible]5 00	45 00–110 00
	121.[illegible]5	3 00–10 00	31.25–111.25
	118 75	5 25–13.75	38 75–113.75
	118 75	3.75–13.75	38.75–113.75
	103 00	3.75–13 00	38 00– 93 00
	116 86		
S.E.	2 45		
[illegible]	128 00	15 00–50 00	55 00–115 00
[illegible]	118 75	10 00–29.25	54 25–113 75

Extravascular volume of distribution, V(ev), was computed as

$$V(ev) \quad V(total) \quad V(iv) \tag{9}$$

where V(iv) denotes intravascular volume of distribution (plasma volume). The distribution volume located in sarcoplasmic reticulum was taken as

$$V(sr) \quad V(total) \; [a(final)/k(final)^2]/Aw(total) \tag{10}$$

where a(final) is the interception with the ordinate at time zero of k(final) (see discussion). Mean transit time of ^{14}C-inulin in sarcoplasmic reticulum, $\bar{t}(sr)$, was taken as

$$\bar{t}(sr) \quad 1/k(final) \tag{11}$$

Results

The experimental results are presented in Tables I, II and III. Two expts. will be considered separately in the discussion (expts. A and B). Mean, x, and standard error of the mean, S.E., are calculated exclusively from expts. Nos. 1 to 7. Mean value of capillary extraction, E, was 0.307 (S.E. 0.077) and mean capillary diffusion capacity CDC, was 0.84 ml/100 g min (S.E. 0.07 ml/100 g min). From an assumed capillary surface area of 70 cm²/g the permeability coefficient, P_d, was calculated to 0.20 10^{-4} cm/s (S.E. 0.02 10 cm/s). Plasma flow as measured directly from the venous outflow was 4.0 ml/100 g min (S.E. 1 1 ml/100 g min). Plasma flow as calculated by analysis was of similar value 4.0 ml/100 g min (S.E. 1 1 ml/100 g min) as mean V(iv) was 2.27 ml/100 g (S.E. 0 12 ml/100 g) and mean t(iv) was 1.03 min (S.E. 0.31 min). The mean transit times in the regions were: t(total) ~ 6.35 min (S.E. 1.62 min), t(ev) 18.82 min (S.E. 1.99 min) and $\bar{t}$(sr) 40 65 min (S.E. 2.95 min). Total volume of distribution was 15.31 ml/100 g (S.E. 2.02 ml/100 g). Extravascular volume of distribution was 13 04 ml/100 g (S.E. 1.92 ml/100 g) of which 9.94 ml/100 g (S.E. 1.95 ml/100 g) was located in sarcoplasmic reticulum (V(sr)/V(ev) ~ 0.76). End time of observation, was in mean 116.86 min (S.E. 2.45 min) with range 103 to 121.25 min. The rate constant of the final slope, k(final), was −0.0255 min⁻¹ (S.E. 0.0021 min). The

TABLE IV Permeability coefficient P_d, for inulin in organs with continuous capillaries.

Tissue	P_d 10^4 (cm/s)	Method	Reference
Muscle	0 50 (0 39)	OT	Pappenheimer Renkin and Borrero 1951 (Perl 197
	0.3	OT	Landis and Pappenheimer 1963
	0 26	ID	Crone 1963 b
	0.09	ID	Trap-Jensen and Lassen 1970
	0 12	ID	Trap-Jensen and Lassen 1971
	0.12	TU	Wittmers, Bartlett and Johnson 1976
	0.20	SI	Paaske and Sejrsen 1977 (present study)
Lung	0.3	TU	Wittmers 1974
	0.4	TU	Wittmers, Bartlett and Johnson 1976
Heart	0.4	TU	Schafer and Johnson 1964
	0.54	TU	Vargas and Johnson 1967
	0.27	ID	Alvarez and Yudilevich 1969
	0.58	OT	Bassingthwaighte, Yipintsoi and Grabowski 1973
	0 51	TU	W ttmers, Bartlett and J hnson 1976
Diaphragm	0 19	TU	W ttmers, Bartlett and Johnson 1976

Abbreviations ID ndicator diffusio method, OT osmotic transient method, SI single injection, exter counting method, TU tissue ptake method

average weight of the muscles used for expts. was 24 6 g (S E. 1 4 g) whereas that of the contralateral control muscles was 25 1 g (S E. 1 8 g) The weights of the expt. muscles and control muscles did not differ significantly as estimated by the Wilcoxon test for paired observations ($p > 0$ 10)

An example of the initial part of an experimental curve with back extrapolation is presented in Fig. 1 (expt. no 7)

Discussion

Application of the single injection, external registration technique to the single inlet, single outlet system of the autoperfused cat gastrocnemius muscle preparation has been discussed elsewhere (Sejrsen 1970 Paaske 1977 c) In the present study the response curve as obtained from ^{14}C inulin activities in venous blood samples was treated analogously from the kinetic principle that heights on the residue curve equal areas on the venous curve.

Table IV lists literature values of P_d in organs with continuous capillaries. When the findings of the present series of a permeability coefficient of 0.20 10^{-4} cm/s for ^{14}C-inulin in the continuous capillaries of skeletal muscle assuming a capillary surface area of 70 cm²/g are related to other studies performed with methods based on indicator diffusion this supports the hypothesis suggested earlier that capillaries of continuous type exhibit similar permeation characteristics regardless of the tissue in which they are located (Paaske 1976) In an earlier study (Paaske 1977 c) it was demonstrated that CDC for hydrophilic molecules is linearly related to plasma flow It was argued that the number of perfused capillaries increases in proportion to the CDC increase. On this basis, the capillary surface area was recalculated at various plasma flows. At a plasma flow of 4 ml/100 g min, as in the present study capillary surface area is probably 20 cm /g which implies a P_d-value for ^{14}C-inulin of ~0.5 10 cm/s.

The free diffusion coefficient in water at 37°C of the ^{14}C inulin employed in the present study D(^{14}C inulin), was found to be 0.221 10^{-4} cm /s (S E. 0.008 10 cm /s, n ~5) using

a technique of diffusion in agar (Sejrsen 1977 unpublished). This corresponds to a Stokes-Einstein molecular radius, a_{s-E}, of 14.8 Å and a molecular radius, a_e, of 15.3 Å when estimated from the formula of empirical viscometric correction $a_e = a_{s-E} \cdot (1 + (0.5/a_s))$ (Schultz and Solomon 1961). The ^{14}C-inulin preparation contained less than 2.7 per cent of the activity in molecules of lower molecular weights. In an earlier study (Paaske 1977 a) the free diffusion coefficients in water at 37°C for ^{51}Cr EDTA and Co-B12 were reported to be 0.700 10 cm²/s and 0.390 10 cm²/s, respectively. From these values it can be calculated that D(^{51}Cr-EDTA)/D(^{14}C-inulin) = 3.17 and D(^{57}Co-B12)/D(^{14}C-inulin) = 1.76. In a study with the same muscle preparation as employed in the present series (Paaske 1977 c) CDC(^{51}Cr-EDTA) was found to be 2.70 ml/100 g min at a plasma flow corresponding to the mean value of the present series (4.0 ml/100 g min). In these expts. CDC(^{57}Co-B1) was determined simultaneously giving 1.71 ml/100 g min. As CDC(^{51}Cr-EDTA)/CDC(C inulin) 3.21 and as CDC(^{57}Co-B12)/CDC(C-inulin) = 2.04 it is concluded that ^{51}Cr-EDTA, ^{57}Co-B12, and ^{14}C-inulin diffuse across the capillary membrane of skeletal muscle at rates proportional to their respective free diffusion coefficients in water *i.e.*, restricted diffusion does not occur for C-inulin compared to the two other indicators.

Since the data did not give evidence of restricted diffusion they can obviously not be used for calculation of an equivalent pore radius from the theory of restricted diffusion. Considering the ariation of the experimental data it would, however seem reasonable to believe that a pore radius of at least 90 Å would be able to account for the present findings. The results are inconsistent with the hypothesis of transcapillary exchange of hydrophilic substances through 30 Å equivalent pores (or 37 Å slit width) as suggested by Pappenheimer Renkin and Borrero (1951). If transcapillary exchange had taken place through 30 Å pores, a CDC(C-inulin) of some 0.27 ml/100 g min would ha e been expected, but this was not the case. Similarly the Karnovsky (1967 1968) interendothelial slit width of 40 Å can be excluded as morphological equivalent for the pore. As previously argued (Paaske 1977 a, b, c) transcapillary exchange of hydrophilic indicators through the intraendothelial channel system of fused vesicles (Simionescu, Simionescu and Palade 1975) could explain the findings of absence of restricted diffusion i tissues with continuous capillaries.

The data of the present series are in agreement with results obtained by an alternative method based on indicator diffusion. Crone (1963 b) reported absence of restricted diffusion to inulin as compared to sucrose using the indicator diffusion method on a dog hindlimb preparation. However Trap-Jensen and Lassen (1971) used the same indicators and method on the exercising human forearm and found massive restricted diffusion which led to a slit width estimate of 40 Å.

Back diffusion of indicator can be defined as early re-entry of indicator from interstitium to blood while the indicator bolus is still positioned in exchange vessels. This phenomenon leads to underestimation of capillary permeability which is seen in expts. A and B (Tables I and II). Fig. 2 show that back-diffusion for ^{14}C-inulin in skeletal muscle has a significant effect when plasma flow is below some 0.75 ml/100 g min.

As calculated from the kinetic analysis the extravascular distribution volume for ^{14}C inulin in skeletal muscle was 13.04 ml/100 g. This value corresponds to the mean value of

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Muscle	0 50 (0.39)	OT	Pappenheimer Renkin and Borrero 1951 (Perl 1971)
	0 3	OT	Landis and Pappenheimer 1963
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	0 09	ID	Trap-Jensen and Lassen 1970
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	0.54	TU	Vargas and Johnson 1967
	0.27	ID	Alvarez and Yudilevich 1969
	0 58	OT	Bassingthwaighte, Yipi taol and Grabowski 1975
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Discussion

Application of the single injection, external registration technique to the single inlet, single outlet system of the autoperfused cat gastrocnemius muscle preparation has been discussed elsewhere (Sejrsen 1970 Paaske 1977 c) In the present study the response curve as obtained from ^{14}C-inulin activities in venous blood samples was treated analogously from the kinetic principle that heights on the residue curve equal areas on the venous curve.

Table IV lists literature values of P_d in organs with continuous capillaries. When the findings of the present series of a permeability coefficient of 0.20 10^{-5} cm/s for ^{14}C-inulin in the continuous capillaries of skeletal muscle assuming a capillary surface area of 70 cm^2/g are related to other studies performed with methods based on indicator diffusion this supports the hypothesis suggested earlier that capillaries of continuous type exhibit similar permeation characteristics regardless of the tissue in which they are located (Paaske 1976). In an earlier study (Paaske 1977 c) it was demonstrated that CDC for hydrophilic molecules is linearly related to plasma flow It was argued that the number of perfused capillaries increases in proportion to the CDC increase. On this basis, the capillary surface area was recalculated at various plasma flows. At a plasma flow of 4 ml/100 g min, as in the present study capillary surface area is probably 20 cm /g which implies a P_d-value for ^{14}C-inulin of ~0 5 10 cm/s.

The free diffusion coefficient in water at 37°C of the ^{14}C-inulin employed in the present study D(^{14}C-inulin), was found to be 0.221 10^{-5} cm /s (S E 0 008 10^{-5} cm /s, n = 5) using

a technique of diffusion in agar (Sejrsen 1977 unpublished). This corresponds to a Stokes-Einstein molecular radius, a_{S-E}, of 14.8 Å and a molecular radius, a_e, of 15.3 Å when estimated from the formula of empirical viscometric correction $a_e = a_S \cdot (1 + (0.5/a_e))$ (Schultz and Solomon 1961). The ^{14}C-inulin preparation contained less than 2.7 per cent of the activity in molecules of lower molecular weights. In an earlier study (Paaske 1977 a) the free diffusion coefficients in water at 37°C for ^{51}Cr EDTA and ^{57}Co-B12 were reported to be 0.700 10 cm /s and 0.390 10 cm /s, respectively. From these values it can be calculated that D(^{51}Cr-EDTA)/D(^{14}C-inulin) = 3.17 and D(Co-B12)/D(^{14}C-inulin) = 1.76. In a study with the same muscle preparation as employed in the present series (Paaske 1977 c) CDC(^{51}Cr-EDTA) was found to be 2.70 ml/100 g min at a plasma flow corresponding to the mean value of the present series (4.0 ml/100 g min). In these expts. CDC(^{57}Co-B12) was determined simultaneously giving 1.71 ml/100 g min. As CDC(^{51}Cr EDTA)/CDC(C inulin) 3.1 and as CDC(^{57}Co-B12)/CDC(^{14}C-inulin) 2.04 it is concluded that Cr EDTA, ^{57}Co-B12, and ^{14}C-inulin diffuse across the capillary membrane of skeletal muscle at rates proportional to their respective free diffusion coefficients in water *i.e.* restricted diffusion does not occur for ^{14}C-inulin compared to the two other indicators.

Since the data did not give evidence of restricted diffusion they can obviously not be used for calculation of an equivalent pore radius from the theory of restricted diffusion. Considering the variation of the experimental data it would, however, seem reasonable to believe that a pore radius of at least 90 Å would be able to account for the present findings. The results are inconsistent with the hypothesis of transcapillary exchange of hydrophilic substances through 30 Å equivalent pores (or 37 Å slit width) as suggested by Pappenheimer, Renkin and Borrero (1951). If transcapillary exchange had taken place through 30 Å pores, a CDC(^{14}C-inulin) of some 0.27 ml/100 g min would have been expected, but this was not the case. Similarly the Karnovsky (1967, 1968) interendothelial slit width of 40 Å can be excluded as morphological equivalent for the pore. As previously argued (Paaske 1977 a, b, c) transcapillary exchange of hydrophilic indicators through the transendothelial channel system of fused vesicles (Simionescu, Simionescu and Palade 1975) could explain the findings of absence of restricted diffusion in tissues with continuous capillaries.

The data of the present series are in agreement with results obtained by an alternative method based on indicator diffusion. Crone (1963 b) reported absence of restricted diffusion for inulin as compared to sucrose using the indicator diffusion method on a dog hindlimb preparation. However, Trap-Jensen and Lassen (1971) used the same indicators and method on the exercising human forearm and found massive restricted diffusion which led to a slit width estimate of 40 Å.

Back diffusion of indicator can be defined as early re-entry of indicator from interstitium to blood while the indicator bolus is still positioned in exchange vessels. This phenomenon leads to underestimation of capillary permeability which is seen in expts. A and B (Tables I and II). Fig. 2 shows that back-diffusion for ^{14}C-inulin in skeletal muscle has a significant effect when plasma flow is below some 0.75 ml/100 g min.

As calculated from the kinetic analysis the extravascular distribution volume for ^{14}C inulin in skeletal muscle was 13.04 ml/100 g. This value corresponds to the mean value of

some 13 8 ml/100 g for inulin in skeletal muscle reported by Crone and Garlick (1970). It is generally assumed that the extravascular distribution volume of inulin is smaller than those of sucrose and ⁵¹Cr EDTA, which have similar permeation and diffusion characteristics (Ogston and Phelps 1961 Page 1963 Law and Phelps 1966, Crone and Garlick 1970). Using the single injection, external registration technique with ⁵¹Cr EDTA as indicator on the autoperfused cat gastrocnemius muscle preparation, Sejrsen (1977 unpublished) found an extravascular distribution volume of 13 1 ml/100 g when kinetic analysis was performed on curves that had been followed for 120 min. In addition, the final slope of this series was $-0\,0285$ min^{-1} for ⁵¹Cr EDTA as compared to the $-0\,0255$ min value for ¹⁴C-inulin observed in the present study For these reasons it must be questioned whether sucrose (⁵¹Cr EDTA) and ¹⁴C inulin have different extravascular volumes. In nephrectomized rabbits, Kruhøffer (1946 a and b) found almost equal distribution volumes for sucrose and inulin Also the disappearance of these indicators from blood took place with rate constants proportional to the respective free diffusion coefficients in water

The multiexponential course of the extravascular transit curve obtained in the present study is indicative of a non-mixed interstitial compartment in skeletal muscle. However the *monoexponentiality of the final slope of the curve indicates a well-mixed compartment within* the interstitial space. The sarcoplasmic reticulum forms lace like sleeves around myofibrils and is in communication with the interstitium outside the cell by the T system of tubules (Porter and Palade 1957) The sarcoplasmic reticulum has about the same volume as the interstitium outside the cell. The longitudinal distance between two Z-bands (one sarcomere length) is about 2 μm for which reason diffusional gradients in the sarcoplasmic reticulum will be eliminated within a few ms. Washout from this system would simulate washout from a well-mixed compartment On this basis it would seem reasonable to assume that the final washout takes place from the sarcoplasmic reticulum. As a consequence of these considerations the distribution volume located in the sarcoplasmic reticulum was calculated in accordance with Eq 10 for ¹⁴C inulin and ⁵¹Cr EDTA As indicated in Table II V(sr) was 9.94 ml/100 g for ¹⁴C-inulin which corresponds to about 76 per cent of V(ev). This figure is somewhat larger than the 6.2 ml/100 g V(sr) value found for ⁵¹Cr EDTA. Consequently it is suggested that the extravascular volumes of distribution for inulin and sucrose *are similar* and that both indicators penetrate into the sarcoplasmic reticulum This hypothesis must, however be examined with other methods, *e.g* autoradiography that permit morphological localization of the radioactive substances.

Supported by the Danish Medical Research Council.

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Acta physiol. scand. 1977 100 446–451

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A Simple Radioenzymatic Procedure for the Determination of Choline and Acetylcholine in Brain Regions of Rats Sacrificed by Microwave Irradiation

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Received 25 February 1977

Abstract

ECKERNÄS, S Å and S M AQUILONIUS. *A simple radioenzymatic procedure for the determination of choline and acetylcholine in brain regions of rats sacrificed by microwave irradiation* Acta physiol. scand. 1977 *100* 446–451

A rapid and sensitive radioenzymatic method for analysis of choline (Ch) and acetylcholine (ACh) concentrations in rat brain regions is described. The ACh content is obtained as the difference in Ch concentration between a hydrolysed and an unhydrolysed tissue sample. The ACh content was 16 ± 1.0 nmol/g in the cortex and 76 ± 2.4 nmol/g in the striatum and the corresponding Ch values were 23 ± 2.5 and 33 ± 1.2

Key word Choline, acetylcholine, determination, rat, microwave.

To date, the methods most frequently used for the determination of acetylcholine (ACh) and choline (Ch) are radioenzymatic (Feigenson and Saelens 1969 Goldberg and McCaman 1973 Shea and Aprison 1973) and gas chromatographic (Hammar *et al* 1968, Hanin and Skinner 1975 Jenden *et al* 1973 Szilagyi *et al* 1972) procedures. The application of gas chromatography coupled with mass-spectrometry (Hammar *et al* 1968, Hanin and Skinner 1975 Jenden *et al* 1973) is probably the most specific method available at present but the extreme cost of the equipment cannot be met in most laboratories. The radioenzymatic procedures for ACh and Ch determination described in the literature (Feigenson and Saelens 1969 Goldberg and McCaman 1973 Shea and Aprison 1973) involve several steps prior to the final analysis—probably due to the very high Ch concentrations obtained in brain tissue from animals killed by other procedures than microwave irradiation. This is time consuming and should increase the total error of the assay

We now describe a modification of the previously used radioenzymatic procedure for Ch and ACh determination based on enzymatic acetylation of free Ch with ^{14}C-acetyl-CoA, using purified choline acetyltransferase (ChAT EC 2.3 1 6) (Feigenson and Saelens 1969 Goldberg and McCaman 1973 Shea and Aprison 1973) After ion-pair extraction of the ^{14}C ACh formed, using tetraphenylboron (Kalignost), directly in the scintillation vial

man 1975), radioactivity is measured by liquid scintillation counting. In one aliquot of the extract endogenous ACh is specifically hydrolysed by means of ACh-esterase and the l Ch concentration of the sample is determined. The concentration of endogenous Ch measured in an unhydrolysed aliquot and the ACh level is then obtained as the difference Ch concentration between the two aliquots. About 120 determinations can be performed per day

Material and methods

Purification of choline acetyltransferase This procedure is based on the methods described by Mannervik & Sörbo (1970) and Shea and Aprison (1973) All processing was performed at temperature below C. 40 g of bovine caudate nuclei were homogenized in 100 ml of solution containing 0 125 M NaCl 1 mM EDTA, using a Potter-Elvehjem homogenizer The volume was adjusted to 150 ml and the pH adjusted to 8 0 by adding 1 M ammonia 5 ml of 1-butanol was added and after 10 min of continuous mixing the homogenate was centrifuged for 20 min at 2 700 g The pH of the supernatant was adjusted 6 0 with 1 M HAc and the supernatant was immediately loaded on 2 5 20 cm CM Sephadex® C 50 column (Pharmacia, Sweden) equilibrated with 10 mM Na-phosphate buffer (pH 6 0) containing 1 mM EDTA The column was eluted with about 750 ml of solution containing 0 125 M NaCl and 10 mM Na-phosphate buffer (pH 6 0) containing 1 mM EDTA, or until all red material had been eluted. A linear gradient from 0 125 M to 1 0 M NaCl in the Na-phosphate-EDTA-buffer (pH 6 0) was applied. The effluent collected in 7 ml fractions and in fractions 15–35 the ChAT activity was determined as described earlier (Eckernäs and Sundwall 1975) The enzyme mostly appeared in fractions 20–28 and the five fractions with the highest activities were pooled. The pH was adjusted to 8 0 with 1 M NaOH and enzyme preparation was divided into 500 µl aliquots which were stored at 90°C The enzyme was stable for at least 10 months The above-described ChAT purification, which can be performed in one day results in an enzyme batch sufficient for about 4 000 determinations of Ch.

Brain samples. Male Sprague-Dawley albino rats weighing 120–240 g were used The rats were killed at the same time of day (11 a m. to 2 p m) by focusing microwave beam on the head for 1 7 (5 kW 2 450 MHz, Gerling-Moore, Inc USA) The brain temperature measured 5 mm below the bregma about after irradiation was 95 C These conditions are known to stop post-mortem changes of brain ACh and Ch (Butcher and Butcher 1974 Guidotti *et al* 1974, Stavinoha *et al* 1973, Schmidt 1976, Modak *et al.* 1976, Nordberg 1977 Weintraub *et al.* 1976)

The parietal cortex and striatum were immediately dissected out and the tissue samples were weighed and homogenized (Ultraturrax®) in 5 ml ice-cold 15 0 1 M formic acid in acetone (Toru and Aprison 1966). After 40 min at 4°C the homogenates were centrifuged at 2 000 g for 10 min The pellet was resuspended 2 ml of 10 0 1 M formic acid in acetone and left for 10 min. After centrifugation as above, the combined supernatants were extracted with 2 5 ml heptanone/chloroform (8 1 v/v) and the aqueous phase was freeze-dried overnight

Enzymatic hydrolysis of ACh The freeze-dried samples were dissolved in 200 µl of 0 5 M Na-phosphate buffer (pH 8 0) 5 µl of ACh-esterase (EC 3.1 1 7 500 units/ml, Sigma USA) was added to 75 µl aliquot, which was incubated for 15 min at 37 C In order to inactivate the ACh-esterase the sealed samples were heated for 60 in boiling water bath

Ch assay 10 µl of sample, 20 µl of reaction mixture and 10 µl of the ChAT preparation were mixed in test tube (Microfuge® LKB-Beckman), to give the following final concentrations 70 µM-(^{14}C)-acetyl-CoA (50 mCi/mmol, NEN), 100 mM NaCl, 50 mM phosphate buffer (pH 8 0), 0 5 mM EDTA 0 2 mM physostigmine salicylate 0–50 µM Ch (originating from samples) 25 nkatal/l ChAT After incubation at 37 C for 60 min the reaction was stopped by adding 30 µl of 1 M HAc 40 µl of the mixture was withdrawn and added to 5 ml of 10 mM Na-phosphate buffer (pH 7 4) in scintillation vial. The ^{14}C ACh formed was extracted into 12 ml of scintillation cocktail (10 ml toluene, 77 mg Permablend III® Packard, 2 ml acetonitrile and 10 mg tetraphenylboron (Kalignost®) as described by Fonnum (1975). The vials were kept in darkness for 12 h and the radioactivity was then measured in liquid scintillation counter (Isocap 300, Nuclear Chicago division) The Ch concentrations were calculated from standard curve (Fig. 1) obtained in parallel with the samples.

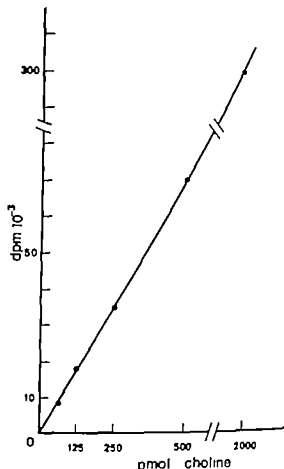


Fig. 1 A typical standard curve. Each point is the mean of two determinations. S.E.M t each point is less than 2 ₒ of the mean, r = 0 99 k = 1 500 ± 2% (± S.D)

Calculation of ACh concent ation After compensation for the 6% dilution caused by addition of the 5 µl of ACh-esterase to the hydrolysed aliquot, the ACh concentration was obta ned as the difference in Ch concentration between the hydrolysed and unhydrolysed aliquots.

All data from the scintillation counter were btained on punched paper tape and mathematical and statistical calculations were performed by a computer

Results

Preparation of ChAT The partly purified enzyme preparation is sufficiently pure for the assay giving a blank value of 2 300 ± 400 cpm (n = 10). Samples from the brain regions normally contain 250–500 pmol (about 13 000–25 000 cpm) Depending on the enzyme batch, 92–98% of Ch in the sample was acetylated after 60 min of incubation at 37°C.

Standard curve In Fig. 1 a typical standard curve (0–500 pmol of Ch) is presented. The same figure shows the linearity up to 2 000 pmol of Ch. The detection limit at 95% confidence level (Hubaux and Vos 1970) of a typical standard curve was about 25 pmol.

Extraction of ¹C ACh. The extraction procedure taking place directly in the scintillation vials, as designed by Fonnum (1975) was found to be a rapid and specific method for extrac-

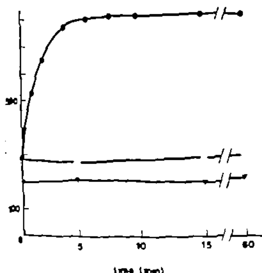


Fig. 2. Free Ch following incubation with AChE for different lengths of time at 37°C, pH 8.0. ●——● striatal homogenate; □——□ striatal homogenate with no AChE added; ▼——▼ solution containing 200 pmol Ch and 2 000 pmol phosphorylcholine incubated with AChE.

tion of ACh. Only 0.5% of acetyl (14C)-CoA was extracted to the toluene phase (~1 000 cpm), while the 14C-ACh formed was extracted with 98% efficiency. It was ascertained by high voltage paper electrophoresis that 14C-ACh was the only radioactive Ch metabolite formed (Appelonius *et al.* 1975).

Control experiments. Fig. 2 shows the increase of Ch following enzymatic hydrolysis of aliquots of a striatal homogenate for different periods of time. Hydrolysis seemed to be complete after only 5 min and no further increase in concentration was seen even after incubation for 60 min. An incubation time of 15 min was therefore used for the routine analysis. No increase in Ch took place in an aliquot with no added AChE or in solution containing 200 pmol Ch and 20 nmol phosphorylcholine incubated with AChE.

When known amounts of Ch and ACh (2.5 nmol of each) were added to aliquots of a brain homogenate and Ch and ACh assays were performed as described above the overall recoveries of added Ch and ACh were about 95 %.

Ch and ACh in rat brain tissue. Our values, shown in Table 1 are in close agreement with those recently reported for rat and mouse brain by other groups using sacrifice by microwave irradiation.

Discussion

As compared with previously employed radioenzymatic procedures (Feigenson and Saelens 1969, Goldberg and McCaman 1973, Shea and Aprison 1973), the method described here has two major advantages. Firstly, due to the low brain Ch levels obtained by the use of microwave irradiation for sacrifice of the animal, it is now possible to estimate the ACh content of the brain from the difference in Ch concentration between a hydrolysed and an

TABLE I Recently published values for Ch and ACh concentration in rats and mice sacrificed by microwave irradiation.

Analytical procedure	Authors	Cortex		Striatum	
		Ch	*ACh* nmol/g ±S.E.M	*Ch*	*ACh*
Radioenzymatic	Present paper	23±2.5 (n=18)	16±1.0 (n=18)	33±1.2 (n=23)	76 4 (n .9)
	Ha brich et al. 1975	17	14	29	83
Gas chromatography	Weintra b et al. 1976	—	16	—	64
	Racagni et al. 1976	—	—	39	51
	Modak et al. 1976[a]	—	26	—	81
	Schmidt 1976	28	28	3.	64
Gas chromatography mass-spectrometry	Butcher & Butcher 1974	—	—	27	98
Bioassay and radioenzymatic	Norberg and Sundwall 1976[a]	31	24	41	75

Mouse.

unhydrolysed tissue sample This obviates the time-consuming separation of ACh and Ch prior to determination. Secondly the isolation of radioactive ACh formed before liquid scintillation counting is highly simplified by the extraction procedure described by Fonnum (1975)

Gas chromatography – mass fragmentography cannot be expected to become available as a routine method for every group studying ACh metabolism. We claim that the present radioenzymatic method is a reasonable alternative combining a high capacity and specificity with a sensitivity allowing Ch and ACh determinations within small regions of the rat brain.

We are indebted to Miss Inger Falk and Miss Lisbeth Lehtipalo for technical assistance. Our thanks are due to Professor B Holmstedt fo kindly allowing us to use the microw ve equipment at the Dept. of Toxicology at Karolinska Institutet, Stockholm. The investigation was supported financially by the S edish Medical Research Council (Project No B75-04X-4373-02).

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Acta physiol. scand. 1977 100 452–456
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Monoaminergic Fluorescence in Frog Skin

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Received 11 March 1977

Abstract

SJÖBERG E *Monoaminergic fluorescence in frog skin* Acta physiol. scand. 1977 *100* 452–456

The Falck Hillarp fluorescence technique was employed in an attempt to determine the distribution of sympathetic innervation in frog skin. No evidence was found of a direct monoaminergic nerve supply to the cells of the non-glandular epithelium in the epidermis. Instead, specific fluorescence was mainly confined to the vicinity of the skin glands. Fluorescent fibers were observed surrounding the mucous type of gland. The secretory content of this gland was not fluorescent. In the granular type of gland the main source of fluorescence was the secretory granules filling the lumen These developed a fluorescence in the spectral range of 5-hydroxytryptamine. The brightness of the fluorescence indicated a very high content of the amine. Fluorimetric analysis showed that no catecholamines were present in the secretion. In glands devoid of secretory granules there were some indications of a monoaminergic innervation of the secretory epithelium, but this was hard to determine because of the abundant nonspecific fluorescence. Sparse dots of specific fluorescence were found close to the surrounding smooth muscle cells. — These findings rule out the possibility of direct sympathetic nervous control of the non-glandular epithelium in frog skin but indicate that this is instead confined to the skin glands.

In a paper by Lang, Sjöberg and Skoglund (1975) a review was given of earlier work dealing with changes in ionic transport and potentials of frog skin induced by nerve stimulation and application of neurohormones. These effects were found to be mediated by adrenergic mechanisms which might either activate mucous skin glands or influence the permeability of the non-glandular epithelium.

Lang *et al* (1975) studied the efflux of Na^+ and Cl^- ions from frog skin during stimulation of sympathetic fibers in the skin nerve and came to the conclusion that the outflow derived mainly from the mucous glands. The innervation of these glands has recently been the subject of a more extensive study using electron microscopic technique (Sjöberg and Flock 1976). Preliminary data from fluorescence microscopic studies and from cholinesterase stainings presented in the same study indicated that the mucous glands very likely received exclusively a sympathetic innervation. This was confirmed in a series of pharmacological experiments (Skoglund and Sjöberg 1977) which also showed that the mucous glands are under β-adrenergic control only Benson and Hadley (1969) reported that α- but not β-adrenergic antagonists blocked the secretory response of the granular type of glands to sympathomimetic stimulation.

However a participation of the non-glandular epithelium in the ionic exchange over the skin during nerve stimulation cannot be ruled out until the innervation of the epidermis has been fully investigated. Whitear (1974) showed that the epidermis of frog skin has a dense innervation and she did not exclude the possibility that some of the nerve fibers supplying the epithelial cell layer might be sympathetic. In fact, Rajerison *et al.* had shown in 1972 that the permeability of isolated frog skin epithelium to Na and Cl ions as well as to water can be increased by catecholamines acting through α- and β-receptors.

In the study to be presented below the innervation of frog skin was investigated by means of fluorescence microscopy in an attempt 1) to determine whether or not the epithelial cell layer may receive sympathetic innervation and 2) to obtain further information on the sympathetic innervation of the mucous and granular glands.

Material and Methods

[illegible] of the species *Rana temporaria* and *Xenopus laevis* were sacrificed by decapitation. Skin samples [illegible] taken from the hind leg, the abdomen and the dorsum. Some pieces were put into [illegible] solution containing [illegible] α-methyl-noradrenaline for 30 min. All samples were then processed according to the Falck [illegible] formaldehyde fluorescence method (Falck *et al.* 1962). They were frozen in propane cooled with [illegible] nitrogen, dried for 40 h, condensed with formaldehyde gas for 1 h [illegible] 80°C and embedded in paraffin. [illegible] of 10 μm were mounted in Entellan (Merck) and examined in [illegible] Zeiss Universal Research micro[illegible] equipped [illegible] BG 12 excitation filter and Zeiss 50, 44 barrier filters. Kodak high-speed Ektachrome [illegible] Gevaert Scopix G films [illegible] ere used for the macrophotographs.

[illegible] microspectrofluorometric analysis (for description of the instrument see Elmqvist *et al.* 1975) [illegible] [illegible] on the secretory contents in the granular glands, recording the emission spectra from mounted [illegible] (cf. [illegible] *et al.* 1975). Granular secretion selectively collected from the skin surface after nerv[illegible] [illegible] was also analyzed by means of fluorometric technique ([illegible] Euler and Lishajko 1961).

Results

[illegible] *glandular epithelium.* Special care was taken to detect fluorescent nerve fibers terminat[illegible] [illegible] on the epithelial cells of the epidermis. On a few occasions fibers developing a weak [illegible] fluorescence specific for monoamines were seen subepidermally against the green background [illegible] fluorescence of the tissue, but these fibers invariably seemed to travel to other structures. [illegible] no indication was found of a direct sympathetic nerve supply to the non-glandular [illegible] epithelium. Whitear (1974) reports of an innervation, revealed in the electron microscope, [illegible] smooth muscle bundles extending through the dermis up to the epidermal epithelium. [illegible] vertically running bundles were easily identified also in fluorescence microscopy [illegible] monoaminergic fluorescence was never observed in the vicinity of them.

Blood vessels received a scant fluorescent innervation and in a few instances varicosities [illegible] observed close to melanophores. The main part of the specific fluorescence was however [illegible] confined to the vicinity of the skin glands.

Mucous glands. Thin nerve fibers, developing a yellow-green specific fluorescence, were [illegible] observed surrounding the mucous type of gland (Fig. 1 A). Clusters of fluorescent vari[illegible] cosities, about 1–2 μm, could also be seen lining the gland's circumference, thus in positions

Acta physiol scand 1977 100 452–456
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Received 11 March 1977

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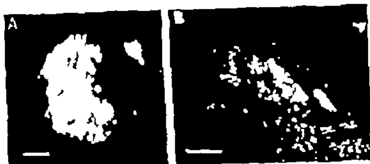


Fig. Granular glands. *A*, Lumen filled with secretory granules exhibiting a very bright yellow fluorescence. *B* Gland devoid of secretory granules, with tiny yellow fluorescent dots spread over the region of the secretory epithelium. Marking in *A* 70 µm, in *B* 50 µm

Discussion

s shown in this investigation the cells of the non-glandular epithelium of frog skin lack monoaminergic innervation, and as a consequence a direct control of the permeability of these cells through sympathetic fibers in the skin nerve can be ruled out. If an adrenergic mechanism similar to that demonstrated by Rajerison *et al.* (1972) on isolated frog skin epithelium should also be operative in the intact epithelium *in situ*, it can thus not be attributable to a direct nervous control. On the other hand, it is quite possible that catecholamines circulating in the blood stream might exert an effect on the epithelium. Another possibility ould be that an excess of transmitter substance released from the nerve terminals close o glands and vessels might indirectly act on adjacent epithelial cells by diffusion. A more speculative hypothesis is that ejected granular secretion products containing 5-HT might influence the non-glandular epithelial cells by penetrating back into the skin. This hypothesis has some support from Pickles's (1957) finding that topical application of 5-HT to frog skin induces depolarization and increases the ionic transport.

As regards the innervation of the skin glands there is good agreement between the findings described above and those reported in the electron microscopic study by Sjoberg and Flock (1976) as far as the mucous glands are concerned, whereas the fluorescence microscopic findings in the case of the granular glands are not quite clear. The electron microscopic investigation revealed a rich intrinsic innervation of this type of gland (*cf.* also Whitear 1974). However, in fluorescence microscopy the amount of non-specific fluorescence, possibly emanating from a protein-containing vehicle in the secretion, in most of the glands emptied of secretory granules made it almost impossible to identify any monoaminergic neuronal structures. However, in a few instances this type of non-specific fluorescent network was not present and the fluorescence then visible within the region of the secretory epithelium was yellow in the same shade as that developed by 5-hydroxytryptamine. Whether this was due to the presence of serotonin-containing neuronal structures or the formation of tiny droplets, predecessors to the fully formed secretory granules, within the epithelial cells could not be determined. Since adrenaline is known to be the transmitter in the frog's sympathetic



Fig. 1 Mucous glands. *A* Two glands surrounded by fluorescent f bers. *B*. Fluorescent varicosities lining outer contour of the gland. Markings 50 μm.

corresponding to those of the nerve terminals previously observed in electron microscopy (Fig. 1 B) The secretion products of the mucous glands did not exhibit any specific fluorescence.

Granular glands The main source of fluorescence in the granular glands was found to be the secretory granules (Fig. 2 A) These had a diameter of up to 10 μm and a very bright, mostly yellow fluorescence. Using microspectrofluorimetric technique this fluorescence was found to have an emission spectrum with a peak at 530–535 nm which is typical of 5-hydroxytryptamine (*cf* Welsh and Zipf 1966). The granular secretion was also analyzed by means of fluorimetric technique (von Euler and Lishajko 1961) in order to find out whether it might contain catecholamines in addition to 5-HT However this proved not to be the case.

Occasionally the fluorescent granules exhibited shades of orange This was probably due to a side reaction of the amines in the process of formaldehyde incubation giving rise to a shift in the maximal emission a phenomenon known to occur when catecholamines are present in high concentrations in a tissue (Jonsson 1971) Some of the granular glands observed were devoid of the secretory granules described above. In a few of these glands tiny dots, developing a yellow fluorescence of the same shade as that induced by 5-hydroxytryptamine were seen all over the region of the secretory epithelium (Fig. ? B), whereas in other instances a network of non-specific fluorescence filled the interior of the gland. After incubation with α methyl-noradrenaline there was an increased fluorescence in certain spots but since the non-specific fluorescence was very abundant it was hard to judge whether this finding was significant or not.

In the comparatively thick smooth muscle layer which surrounds the epithelium bright yellow-green dots were occasionally seen but never any fluorescent fibers.

Sections were made of skins from the two species *Rana temporaria* a frog considered fairly harmless, and *Xenopus laevis* which is known to have a particularly noxious skin secretion. The latter species generally had larger glands of both types and also a greater number of granular glands. The brightness and appearance of the secretory granules were however the same in both species.

Acta physiol. scand. 1977. 100. 457–470

From the Department of Physiology, Karolinska Institutet, Stockholm, Sweden

In Vitro Studies of Frog Mucous Glands

By

C. R. SKOGLUND and E. SJÖBERG

Received 21 February 1977

Abstract

[illegible]OGLUND, C. R. and E. SJÖBERG. *In vitro studies of frog mucous glands.* Acta physiol. scand. 1977. 100. 457–470.

[illegible] ionic outflow, mainly consisting of Na[illegible] and Cl[illegible] from the mucous glands in an excised nerve-skin preparation of frog has been determined by recording the conductance changes occurring in a fluid layer covering a small area of the skin surface. In the main series of experiments the glands were activated by stimulation of sympathetic nerve fibers in the skin nerve. The relationship between the ionic outflow and the number of nerve volleys was studied over a wide range. The outflow per impulse was found to be fairly constant during the first tens of impulses but diminished gradually with increasing number of stimuli to a certain minimum value—varying in different preparations—after which the outflow ceased completely. During the initial phase of stimulation the outflow is most likely caused by an ejection of preformed secretion due to the contractions of the glandular myoepithelium. The continued outflow in the later stages of the stimulation periods must be due to production of new secretion. Since the glandular epithelium is devoid of nerve terminals a nervous control of the ionic secretion can only be explained by an indirect influence mediated either by transmitter diffusion from the myoepithelial nerve endings or by a close functional coupling between the contractile and the secretory gland cells. Adrenaline and noradrenaline induce ionic outflows which like those evoked by nerve stimulation are inhibited by the β-adrenoreceptor blocker propranolol, α-adrenoreceptor blockers being without effect. A seemingly spontaneous finding of tonic changes in the frog skin during nerve stimulation is also described.

In an earlier paper (Lang, Sjöberg and Skoglund 1975) a review was given of previous work by other authors dealing with the effects on frog skin permeability produced by skin nerve stimulation and by application of neurohormones. It appeared that there were two possible ways by which these effects might be produced, viz. either by an action on the non-glandular epithelium or by an action on the mucous glands. To establish whether the first or the second alternative holds true it was found to be necessary to approach the problem by physiological as well as morphological methods. For the physiological analysis a method was developed which allowed determination of the ionic outflow from the skin by measuring the concomitant conductance changes occurring in a fluid layer covering the skin. Application of this method in a previous series of experiments led to the conclusion that the main outflow of ions during sympathetic nerve stimulation was due to an activation of the mucous glands (Lang et al. 1975). A participation of nonglandular epithelium could however not

nervous system (Östlund 1954) it might have been anticipated that this substance would b[e] present in the granular secretion in addition to 5-HT as the secretory process implies [a] breakdown of the richly innervated epithelial cells. The finding that adrenaline is not presen[t] might however be accounted for if instead another substance, e.g. 5-HT should actuall[y] be the transmitter in these fibers. It has not been within the scope of this investigation t[o] determine the identity of the transmitter involved, but in this context it is noteworthy tha[t] Martin and Barlow (1972) could demonstrate an uptake of H 5-HT by the nerve fiber[s] supplying the secretory tubules of the salivary gland of *Octopus*

This work was supported by a grant from the funds of Karolinska Institutet.

I wish to express my gratitude to Professor Kjell Fuxe and Assistant Professor Gosta Jonsson, Department of Histology Karolinska Institutet, for valuable advice and technical assistance.

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be excluded until it had been ascertained whether or not the nonglandular epithelium is sympathetically innervated.

Morphological studies of the frog skin were therefore performed by light and electron microscopic techniques (Sjoberg and Flock 1976) as well as by fluorescence microscopy according to Falck–Hillarp (Sjöberg 1977) The latter investigation established that the nonglandular epithelial cells were devoid of any fluorescent nerve endings. Fluorescent varicosities were however found in clusters along the circumference of the mucous gland. Electron microscopy revealed that these nerve terminals were situated close to the myoepithelial layer It was also found that the glandular secretory epithelium lacked innervation. These new data on the innervation of the mucous glands have to be taken into consideration in the physiological analyses of their secretory mechanisms The secretion induced by nerve stimulation has since long been known to involve ejection of preformed mucus due to the contraction of the myoepithelium (Engelmann 1872) whereas it is still an open question whether there is also a nervous control of the glandular epithelial cells.

In the present paper we have tried to illuminate this question by analyses of the ionic outflow from the mucous glands under varying stimulus conditions. Special attention has been paid to the relationship between the ionic efflux and the number of sympathetic nerve volleys delivered at a given frequency Effects of application of neurohormones and blocking agents will also be reported. Finally attempts to record the volume of the secretion induced by nerve stimulation will be described

Methods

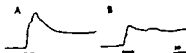
Material. Frogs of the species *Rana temporaria* and *Rana esculenta* were used in most of the experiments but in a few of them lso *Xenopus laevis*. A piece of calf skin was dissected out with the ttached cutaneous branch of the sural nerve together with the sciatic nerve trunk.

Stimulating and recording arrangements. A Grass S4 stimulator was connected via an isolation unit to pair of stimulus electrodes (5 mm apart) pplied to the sciatic nerve trunk which was lifted out of the Ringer solution during stimulation. The timuli were recorded on one channel of a Grass polygraph, other channels being used for monitoring of conductance and fluid level. The timulus markings in the original recordings were converted into drawn ndications the f gures.

Conductance measurements The ski was mounted n a two-compartment perspex chamber (cf Fig 1 in Lang *et al* 1975) with the corium side downwards n contact with Ringer solution n the lower compartment The outer surface of the skin forms the bottom of the upper test compartment which has a volume of 0.5 ml and is filled with distilled water from the beginning of the experiment. A conductance probe is placed close to the skin surface. It consists of two thi platinum electrodes between which high-frequency field (1 085 MHz) is applied from a high-frequency reflectometer (Haapanen 1962). The conductance changes are reflected as variations n absorption of the high-frequency field and this is recorded by the reflectometer as changes in its grid current value (I_g) which can be read on an ammeter nd also continuously recorded n one channel f the Grass polygraph.

Chemical analysis of the fluid in the test chamber has shown that the main ionic outflow consists of Na^+ and Cl^- in equal amounts, the outflow of other ions being negligible. Hence the recorded increase in conductance can with a certain approximation be converted nt an increase in NaCl concentration, expressed in μM. The μM values were read off from diagram showi g the relation between the I_g value and the NaCl concentration, obtained by calibration of the probe-reflectometer against solutions of known NaCl content. Knowing the fluid volume in the test chamber the outflow of NaCl in μg can then be calculated Both expressions will be used i the following.

For further details of the technique we refer to the paper by Lang *et al.* (1975), in which the errors involved in the measurements are also discussed. An additional comment to the previous description is however



:1 Typical differences in conductance recordings obtained without stirring (*A*) and after stirring (*B*) of fluid in the test chamber. See text. *Below*: stimulus indications (10 Hz during 10). The increase NaCl concentration 40 μM in both cases. The original stimulus markings in this and following ures showing conductance records converted into draw lines.

ment. As illustrated in Fig. 4 and 5 in the paper referred to and as illustrated here in Fig. 1 *A* the conduct e recording may show peak reflecting the initial high ionic concentration at the probe placed close he skin surface at the bottom of the chamber before diffusion int the rest of the fluid has taken place al volume 0.3–0.5 ml). A faster equilibration can be attained (*cf.* Fig. 1 *B*) by stirring the fluid by all jet of air hich sets the fluid in rotation. This as utilized in some of the experiments in others the lution time could be shortened by reducing the fluid to small layer barely covering the probe.

The frog calf skin contains about 50 mucous glands per square mm. The skin area exposed in the test mber of 63 mm² can thus be estimated to contain around 3 000 glands. It can, however, not be excluded t the mounting of the skin in the perspex chamber might have damaged some of the nerve branches plying the exposed skin area, and the actual number of glands ith intact innervation might therefore less. In the skin of the hind leg there are also number of granular glands. These are activated simul-neously during nerve stimulation, but their secretory content is more or less completely emptied after seconds of stimulation and ill not be restored within the time course of our experiments. The granular cretion was rinsed out by repeated changes of the test chamber fluid before the actual experiments started.

Pharmacological experiments. The following drugs were administered to the fluid in the test compart nt: atropine, noradrenaline, acetylcholine, isoproterenol, propranolol, phentolamine nd Substance P mmercially available, Beckman). Most of these agents were nonionized and did not interfere with the nductance measurements. Some ionized substances ere also applied taking care to use concentrations r enough to ensure that the conductance of the test solution did not increase to such an extent that the nsitivity of the method as affected.

Recording of the fluid level in the test chamber. A method devised by Haapanen was used to record mable changes in the fluid level of the test chamber due to an outflow of etery secretion concomitant h the ionic effect. The transducer consisted of piece of tungsten ire ith long tip of almost uniform meter of about 25 μm (obtained by etching in KNO_3 using V at 50 Hz). This uninsulated tip was lowered vertically into the fluid of the test compartment and connected to Tektronix type 130 L–C ter, set to measure the capacitance between probe tip and fluid. The changes in fluid level ere found to proportional to the changes in capacitance and were recorded on one channel of the polygraph. The thod allowed of measuring level changes down to 1 μm.

Results

nductance measurements of the ionic outflow

rve stimulation

ually the outflow in response to a single nerve stimulus is too small to be recordable, t in preparations in good state this may sometimes be possible, as illustrated in Fig. 2 *A* owing a conductance change representing 20 μM increase in NaCl concentration of the t chamber fluid. This response although small, represents in fact the maximal outflow m this preparation following single nerve volley: since supramaximal stimulus strength 0 V) was used, all sympathetic fibers were stimulated and a maximal number of glands tivated. If, instead, a stimulus strength in the threshold region (4–5 V) had been used me nerve fibers might have failed to respond resulting in reduced outflow.

Records B–D show the successive increase in outflow when stimulus trains consisting of

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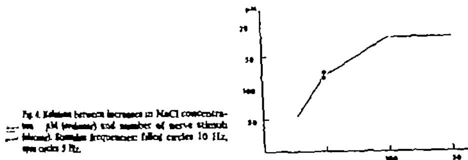


Fig. 4. Relation between increases in NaCl concentration (μM (ordinate)) and number of nerve stimuli (abscissa). Stimulus frequencies: filled circles 10 Hz, open circles 5 Hz.

The data obtained by stimulation at 10 Hz show that in this preparation there was an increment in outflow with increasing number of impulses up to 100. However, the relationship is not linear: the increase in outflow between 25 and 50 stimuli is 3 μM per impulse as against only 1 μM between 50 and 100 stimuli. Whether the relatively smaller outflow during longer stimulation periods is due to a complete cessation of secretion in some of the glands or to a reduced outflow in the individual glands—or a combination of both—is not possible to deduce from the present type of experiments. It is apparent from the diagram that an increase of the stimulation period to 15 s did not result in any further increase in ionic outflow, which implies that after about 10 s of stimulation the outflow from all glands had completely ceased.

The number of stimuli after which such a complete fatigue sets in varies in different preparations. Thus, in another preparation there was a continued outflow up to about 250 stimuli, as illustrated by the conductance records in Fig. 5 and the plottings of the results from this experiment in Fig. 6. The trend of the curve is the same as in the previous experiment, the outflow per impulse being largest during the shortest stimulation period, from 2.5 to 5 s, then being successively diminished. Prolongation of the stimulation periods from 25 to 100 s, corresponding to a range from 250 to 1 000 stimuli, did not result in any further significant efflux.

Occasionally, however, the outflow was observed to continue up to 120 s on stimulation at 10 Hz, as illustrated in Fig. 7 (filled circles). Again the largest outflow per impulse can be seen to occur during the shortest stimulation periods, 10–20 s. The exact stage at which the complete fatigue set in in this preparation was not determined.

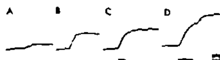


Fig. 5. Conductance records showing increases in ionic concentration expressed in μM NaCl, obtained due to stimulation periods of varying durations (frequency 10 Hz). A, 2.5 s, 25 μM ~ 7.5 nmol ~ 0.44 μg NaCl; B, 5.0 s, 85 μM ~ 25.6 nmol ~ 1.49 μg NaCl; C, 10.0 s, 120 μM ~ 36.2 nmol ~ 2.10 μg NaCl; D, 15.0 s, 165 μM ~ 49.8 nmol ~ 2.89 μg NaCl.

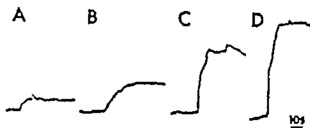


Fig. 2. Conductance records showing increases in ionic concentration in μM NaCl, obtained in response to increasing numbers of stimuli applied at 3 Hz in each stimulus train. The records were obtained with somewhat different sensitivity ranges of the conductance meter and the proportions are therefore not directly comparable. This applies also to Fig. 5 and 9. *A*, 1 stimulus, 30 μM. *B*, 3 stimuli, 55 μM. *C*, 5 stimuli, 70 μM. *D*, 10 stimuli, 115 μM.

3, 5 and 10 stimuli were applied. It is obvious that a summation occurs on repeated stimulation. The responses to the different numbers of stimuli applied in this experiment are plotted in the diagram in Fig. 3, from which it appears that the response is linearly proportional to the number of stimuli within the range from 1 to 10 impulses.

In order to investigate if this relationship holds also over a wider range, a series of systematic investigations was performed. The rate at which the stimuli were delivered in the experiment in Fig. 2 was 3 Hz, but for several reasons it proved to be preferable to choose a somewhat higher stimulus rate when a greater number of impulses were applied. On the basis of an explorative series of experiments in which stimulus frequencies of up to 30 Hz were used 10 Hz was chosen as being most suitable. In most preparations a prompt response was invariably obtained at this frequency which at the same time may not be too high from a physiological point of view. The diagram in Fig. 4 summarizes the results from an experiment in which the number of impulses was varied over a range from 25 to 150. The filled circles represent values obtained by stimulation at 10 Hz. For comparison, some stimulations were also performed at 5 Hz (open circles). The somewhat smaller effect following the same number of impulses when delivered at the lower frequency was a typical finding indicating that the summation was less effective at the longer stimulus intervals.

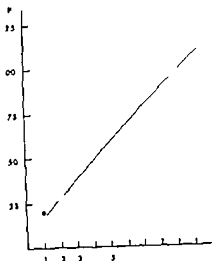


Fig. 3. Plotting of data obtained in experiment in Fig. 2 (using 1–10 stimuli), showing relationship between increase in NaCl concentration in μM (*ordinate*) and number of nerve stimuli (*abscissa*). Some values represent means of two observations.

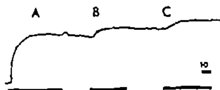


Fig. 8. Conductance record showing recovery from fatigue after short periods of rest. *A*: Stimulation at 10 Hz during 70 s, resulting in outflow of 0.75 µg NaCl, cessation of outflow after about 45 s. *B* and *C*: Renewed stimulations after 30 and 40 s of rest, resulting in outflow of 0.15 and 0.20 µg NaCl respectively.

from the conductance record the outflow had ceased completely after about 45 s of stimulation. However, as seen by the steps in the plateau level on renewed stimulation after 30 and 40 s of rest respectively, a recovery has occurred within the first tens of seconds after the end of stimulation.

A remarkable feature of the nerve–skin preparation is its ability to function for long time periods after it has been dissected out and mounted in the chamber, in which its only oxygen supply is that obtained by diffusion from the air into the Ringer solution. We have had experiments going on for some 6–8 h in which only minor variations in efflux were seen if sufficient rest periods were allowed. On the other hand, it is obvious that fatigue phenomena may set in after a smaller number of impulses at the end than at the beginning of such long term experiments. Preparations kept overnight in the refrigerator at 4 C may respond to stimulation even the following day but it is obvious from the gradually diminished responses in a continued experimentation that a certain deterioration has taken place.

Pharmacological experiment

In an initial series of experiments drugs (cf. Methods) were administered to the Ringer solution bathing the corium side of the skin. However, due to the slow diffusion of the substances across the connective tissue the conductance changes in the solution set in very slowly or were of low amplitude, and hence they were difficult to distinguish from the spontaneous changes which may occur during nonstimulation periods (*cf.* Table I in Lang *et al.* 1975). If higher concentrations of the drugs were used, the diffusion time was reduced but on the other hand the risk of anesthetic effects on the skin nerve submerged in the solution increased. In the main series of experiments the drugs were instead added to the fluid covering the epithelial surface of the skin. The diffusion was then much faster, resulting in prompt effects as illustrated by the curves in Fig. 9, in which *A* shows a recording of the ionic outflow from the skin following the application of adrenaline in a concentration of 5 µg/ml in the test compartment. After a latency of about 10 s a relatively rapid increase in conductance occurs which ends in a plateau in a similar way as during nerve stimulation. The ionic content of this outflow was comparable to that obtained after 250 stimuli in the experiment illustrated in Fig. 5. Higher concentrations of adrenaline resulted in larger outflows which might be maintained for long periods of time. Sometimes the outflow could continue for an hour or more without being reduced, possibly due to cellular damage causing an irreversible increase in permeability.

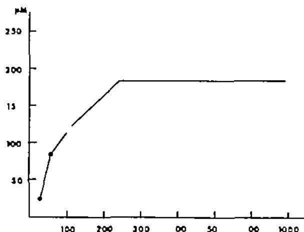


Fig. 6. Plotting of results from experiment illustrated in Fig. 5 showing relationship between increases in NaCl concentration in μM (*ordinate*) and number of nerve stimuli (*abscissa*). Stimulus frequency 10 Hz. The horizontal part of the curves is dra at the mean (175 μM) of the values obtained between 250–1 000 stimuli.

The open circles represent data from an experiment on a rat salivary gland (Thulin 1976) which will be commented on in the Discussion.

The results have so far been expressed as increase in NaCl concentration in μM which is adequate in comparisons of relative changes in outflow in one and the same experiment. However when comparing the results from different experiments, in which the fluid volume in the test chamber may have varied, it is necessary to calculate the absolute values of the outflow in μg NaCl Such calculations were performed on data from experiments on 20 frogs, in which the outflows during stimulation periods from 10–60 s had been determined. The average outflow per cm skin area during 10 s of stimulation at 10 Hz (*i.e* 100 stimuli) was found to be 1.25 μg NaCl, the individual values varying from 0.25 to 3 75 μg.

In all experiments in which quantitative determinations were made a resting period of 5 min was allowed between the stimulations. Even with stimulation periods of 60 s this resting period is usually sufficient to maintain the efflux at a constant level on renewed stimulations. However there is no doubt that after very long pauses, of half an hour to an hour further recovery occurs, since after such long periods of rest the responses to the first stimulus are invariably significantly larger than after the 5-min pauses. This agrees also with the observation that the first response at the beginning of an experiment, immediately after the preparation has been mounted in the chamber is always strikingly large The recovery is a process that seems to start very soon after cessation of stimulation Fig. 8 is from an experiment in which the nerve stimulation was maintained during 70 s at 10 Hz. To judge

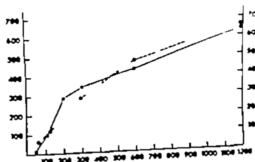


Fig. 7 *Filled circles*. Data from frog mucous gland showing the relation between increases in NaCl concentration in μM (*left ordinate*) and number of nerve timuli (*abscissa*). Stimulus frequency 10 Hz. *Open circles*. Thulin's (1976) data from rat parotid gland showing the relation between olume of secretion in μl (*right ordinate*) and number of nerve stimuli (*abscissa*). *Cf* Discussion.

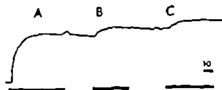


Fig. 8 Conductance record showing recovery from fatigue after short periods of rest. *A*. Stimulation at 1 Hz during 70 s, resulting in outflow of 0.75 µg NaCl, cessation of outflow after about 45 s. *B* and *C*. renewed stimulations after 30 and 40 s of rest, resulting in outflow of 0.15 and 0.20 µg NaCl respectively

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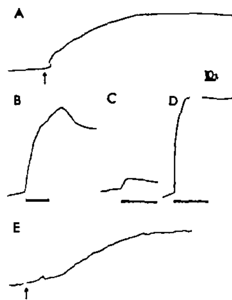


Fig. 9 Conductance records showing changes caused b application of various substances in test chamber *A* Adrena-line solution, 5 μg/ml (*at arrow*) NaCl outflow 3 μg Tm scale refers also to *B–E*. *B–D* Responses to nerve stimulatio before, 10 min after administration of β-blocker propranolo (10 μg/ml) and after rinsing of the preparation respectively NaCl outflow in *B* 2.5 μg; *C* 0 45 μg; *D* 2.10 μg. *E*. Substanc P solution, 0.1 μg/ml (*at arrow*) NaCl outflow 2.8 μg.

No difference was found between the effects of adrenaline and noradrenaline Acetyl-choline was also applied but caused no detectable ionic outflow which rules out any cholin-ergic mechanism participating in the secretion process.

Application of the β-adrenoreceptor stimulating agent isoproterenol in a concentration of 40 μg/ml induced an outflow comparable to that caused by catecholamines. The β-blocking agent propranolol (5–10 μg/ml) inhibited the secretory response to catecholamines as well as to nerve stimulation. The latter effect is illustrated in Fig. 9 *B–D* in which *B* shows the initial large ionic outflow and *C* the reduced response to stimulation 10 min after application of propranolol This blocking effect was reversible, as shown by the restored outflow in *D* after rinsing of the preparation The α-blocking agent phentolamine was ineffective.

Substance P was also tried since it had been found in significant amounts in mammalian exocrine glands and is known to induce sweat secretion in humans (Pernow personal communication) As shown by Fig. 9 *E* application of 0 1 μg/ml of this substance caused an ionic outflow which successively decreased after about 100 s and altogether amounted to 2.8 μg NaCl. This effect is most likely due to myoepithelial contraction (*cf* Skoglund and Sjöberg 1977). Since immunohistochemical investigations have not revealed the presence of Substance P in frog skin (*Hökfelt, personal communication*) the effect might not have any physiological significance.

In the pharmacological experiments performed no attempts were made to establish dose-response relationships.

Recording of fluid level in test compartment

The relation between the amount of NaCl and the watery content of secretion has been established by chemical analyses of the secretion from the mucous glands during neuro-hormonal activation (*e.g.* Campbell *et al* 1967) Provided that this relationship is the same in the secretion induced by electrical nerve stimulation it ought to be possible to calculate

the volume of the secretion from the skin area studied in our experiments on the basis of our data on the ionic outflow. The analyses of the mucous secretion showed that the content of Na^+ and Cl^- ions is only slightly lower than that of plasma, *viz.* 80–95 mM Na^+ and 65–70 mM Cl^- as against 100 mM and 85 mM respectively for plasma. Our own experiments indicated an outflow of Na^+ and Cl^- ions in equal amounts (Lang *et al.* 1975). Assuming, as an approximation, a NaCl concentration of 80 mM in the secretion this would imply that one liter of secretion should contain 4 675 mg NaCl. A secretion amount of 1 μl would then contain about 4.7 μg NaCl. An outflow of 1.5 μg NaCl, which is an average value during 10 s of stimulation at 10 Hz, would thus correspond to a secreted volume of about 0.3 μl.

If a secretion volume of this size is added to the fluid in the test chamber it would cause an elevation of the fluid level of 4.5 μm. Since a method sensitive enough to record changes in fluid level of this magnitude was available it was tempting to perform some measurements. These proved that an increase in fluid level of a size corresponding to the calculated changes did indeed occur. However, the results were in many ways puzzling, and an extensive series of experiments had to be carried out before it could be ascertained that the main cause of the recorded changes in fluid level was not the transfer of water from the inside of the skin to the outer medium, but tension changes in the skin induced by the sympathetic nerve stimulation. It seems worthwhile to publish a few records demonstrating this unexpected finding. A typical record obtained in these experiments is seen in Fig. 10 *A*. After a latency of a few seconds there is a slow, somewhat stepwise rise to a plateau which is reached after some 40 s of stimulation and maintained throughout the stimulation period of 200 s. This plateau represents an elevation of the fluid surface in the test chamber of 7.5 μm, which would correspond to a volume increase of about 0.5 μl.

However, the curve exhibits certain characteristics which speak against its correlation with secretion. Most striking is the return of the level to and even somewhat below the baseline after cessation of stimulation. This tendency was even more pronounced in other experiments, as shown in record *B* in which a shorter stimulation period (25 s) was used. This record is from a later experiment in which the crucial test was performed which revealed the actual cause of the level changes: the preparation was turned upside down so that, instead of the epithelial side, the nonsecreting corium side of the skin formed the bottom of the compartment. A curve identical with that in *B* was then obtained, as shown in record *C*. The obvious conclusion to be drawn from this result is that a water transfer cannot play any noticeable role in the recorded changes in fluid level. The main cause of the level change is instead an increase of the tension of the skin. When mounted in the chamber in the usual way the skin is always somewhat slack, and an increase in tension will result in an elevation of the fluid covering it, whichever side of the skin is facing upwards toward the probe.

There are different contractile elements in the skin that may contribute to such tension variations. Thus a contraction of the myoepithelium of the mucous glands which reduces the outer size of the gland (Skoglund and Sjöberg 1977) may give rise to tension changes in the surrounding connective tissue. The smooth muscle contraction of the granular glands might also contribute to the recorded tension changes. Some authors have described smooth muscle fibers running in oblique direction through the subcutis up to the epithelial layer

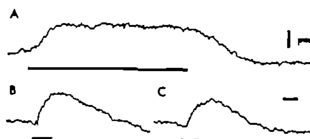


Fig. 10. Tension changes in frog skin during sympathetic nerve stimulation as reflected in changes in fluid level in test chamber. *A*. Maintained increase in tension throughout stimulus period of 200 s. *B*. Tension increase during shorter stimulus period of 25 s. *C*. Same result as in *B* obtained after reversal of skin in test chamber. See text.

(Whitear 1974). An activation of these fibers by the sympathetic nerve stimulation might be the main reason for the recorded effects. Whether this unexpected tension change in the excised skin during nerve stimulation may be of any functional significance under physiological conditions *in vivo* cannot as yet be determined.

Discussion

The sources of error involved in the conductance measurements were discussed in detail in a previous paper (Lang *et al.* 1975), *i.e.* the possible error introduced by relating the conductance change to Na and Cl ions only, when other ions such as K and SO occurring in small amounts in the secretion may also have contributed to the conductance change. However, our aim has not been to analyze the ionic content of the secretion in detail, which can only be made by chemical methods. Our approximation, relating the conductance to NaCl only, has been performed in order to get a numerical index of the total efflux convenient to use in comparisons of ionic outflows under varying stimulus conditions.

It is noteworthy that the skin area under study contains several thousand glands, and even if part of them are not activated due to damage of nerve branches during the dissection, the preparation contains a very large number of units.

One drawback of a multi-unit preparation is that it is difficult to determine whether an increase in ionic efflux is due to a recruitment of new glands or to an increased outflow from the different glands already activated. However, it is reasonable to assume that with a maximal nerve stimulation a relatively constant number of glands is activated by each single stimulus, hence the summation effects observed after a train of stimuli may rather be due to summation effects in the individual glands. Summation of hyperpolarizing responses in single glandular cells during repeated stimulation has recently been demonstrated in the salivary glands of the cockroach (Ginsborg and House 1976).

Engelmann's (1872) observations on individual glands in the toe web made it clear that one immediate effect of nerve stimulation is a contraction of the myoepithelium resulting in an ejection of the glandular content. As was also noticed by Engelmann, and has been verified by us (Skoglund and Sjöberg 1977), a single nerve stimulus is not sufficient to evoke a full contraction of the gland; this requires a train of several impulses. The larger outflow in response to trains of stimuli as compared to the response to a single stimulus (*cf.* Fig.) is most likely due to a more effective expulsion of glandular content on repeated stimulation. However, after some ten stimuli the glands are completely contracted and probably most

of the preformed glandular content has then been expulsed. The fact that during continued nerve stimulation there is a further increase in ionic efflux, most pronounced up to 100–200 impulses, must imply that nerve stimulation does not only elicit contractions but also initiates and maintains ionic secretion. Actually Engelmann described changes in the shape of the glandular epithelium concomitant with the myoepithelial contractions during nerve stimulation.

Further support for the concept of an influence on the secretory epithelium during prolonged nerve stimulation is provided, e.g by Lindley's work of 1969 in which he describes long-lasting changes in impedance and short-circuit currents of frog skin during nerve stimulation. Such changes cannot readily be explained by myoepithelial contractions but must be due to permeability changes in the glandular epithelium.

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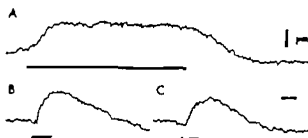


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In Lindley's work and in other investigations, including our own (Lang et al. 1975), it has been taken for granted that the secretory epithelium is innervated. However Sjöberg and Flock (1976) in their electron microscopic studies, have now been able to demonstrate that no nerve terminals are present in the glandular cells but only at the outer surface of the myoepithelium. In view of these findings the mechanisms of the nervous control of the glandular epithelium will have to be reconsidered. The most plausible explanation would be that the transmitter liberated at the terminals close to the myoepithelium diffuses across the cell layer to the glandular cells. A prerequisite for such a process is the close contact between the two types of cells demonstrated in the electron microscopic studies. Such a diffusion should also be facilitated by the comparatively slow breakdown of the transmitter in a cold-blooded animal such as the frog.

The possibility of an electrical transmission from the myoepithelial to the secretory epithelial cells must also be considered in view of the close connection between their cell membranes. In contrast to smooth muscle cells the myoepithelial cells lack sarcolemma. Before any definite conclusion can be drawn concerning such a transmission, more knowledge is required about the electrical properties of the myoepithelial cells. However between adjacent secretory cells zonulae occludentes have been demonstrated (Sjöberg and Flock 1976) which indicates that a close electric coupling may exist between these cells. Similar close connections between cells resulting in low junctional membrane resistance have previously been described in salivary gland epithelial cells (Wiener et al. 1964, Loewenstein et al. 1965).

In mammalian salivary glands a nervous control of the secretion has been established in many investigations. Scott and Pease (1959) demonstrated the presence of nerve terminals between the secretory epithelial cells of the rat parotid gland. The same gland was used by Thulin (1976) in studies of the secretion volumes during parasympathetic stimulation, in which the nerve was stimulated during periods of 60 s using frequencies varying from 1 to 10 Hz. This procedure is different from that applied in our experiments, in which the frequency was kept constant and the stimulation periods were varied, but the range of the number of stimuli used (from below 100 to 1 200) is the same as that studied by us, e.g. in the experiment in Fig. 7. When plotting the data on the secretion amounts given in Thulin's Table I against the number of impulses, as we have done in Fig. 7 above, the relationship proves to be strikingly similar to that found for the ionic outflow in our own

experiments. In view of the obvious differences between the two types of experiments we are not prepared to draw any too far reaching conclusions, but the similarity in quantitative outflows over the wide stimulus range up to 1 200 impulses—in our case determined as ionic content in Thulin's case as volume—seems to be a further support for the concept of a nervous control of the secretory epithelium in the frog mucous gland.

From the studies of the relationship between nerve stimulation and ionic efflux it appeared that the concept of an outflow proportional to the number of stimuli did not hold above some tens of stimuli. Instead, there is a successively decreasing outflow with increasing number of impulses. There are several possible reasons for this fatigue. Thus, e.g. an increase in stimulus threshold of the sympathetic fibers has to be taken into account considering the longlasting positive afterpotentials characteristic of C-fibers which sum up even at the comparatively low stimulus frequencies used (Erlanger and Gasser 1937). However the suprathreshold stimulus strength used ought to be sufficient to compensate for this effect

Another possibility might be a decrease in transmitter outflow Data on the catecholamine release in relation to the number of stimuli applied to sympathetic nerves do not seem to be available concerning frogs, but studies on mammalian preparations have shown that the secretion of tracer noradrenaline per stimulus tended to decline after about 120–150 s of stimulation at 10 Hz (Stjärne and Wennmalm 1970 Stjärne 1974) Apart from the possibility of species differences, the liberation of transmitter from the nerve terminals might be more easily affected by repeated stimulation in an *in vitro* preparation like ours, with no special arrangements for oxygen supply Thus a decrease in transmitter outflow cannot be excluded as a factor in the fatigue.

Finally there is the possibility of a diminished response from the glandular cells proper Since very little is known about glandular secretion mechanisms one can only speculate. The possibility exists, however that the glandular cells may fail to restore their NaCl content as quickly as it is released. The fact that a pronounced increase in outflow has been observed after long resting periods of 0 5 to 1 h or more might indicate that a loading up actually takes place during rest Speaking against the alternative that the fatigue should be due to a lack of response of the glandular epithelial cells is the occasional observation that application of adrenaline caused an ionic outflow also from fatigued preparations which did not respond to nerve stimulation. This reasoning holds however only if the glands influenced by the topically applied adrenaline are identical with those previously activated by the nerve stimulation and are not glands that might have been deprived of innervation during dissection and hence not stimulated.

An influence on the nonglandular epithelium cannot be excluded when active substances are applied on the epithelial side of the skin but since the recorded ionic efflux in the pharmacological experiments was of about the same magnitude as that induced by nerve stimulation we assumed that it emanated mainly from the glands. The prompt response to adrenaline indicates that its primary site of action is on the myoepithelium which is caused to contract and squeeze out a certain amount of ionic secretion. This view is also supported by the relatively short duration of the outflow when adrenaline was applied in moderate concentrations. *In vivo* observations on the toe web glands have revealed that adrenaline elicits

powerful contractions of the myoepithelium (Skoglund and Sjoberg 1977). The longlasting ionic outflow caused by higher adrenaline concentrations may on the other hand, be attributed to an action on the glandular epithelium.

In more differentiated glands, such as the dog's submaxillary gland, the secretory cells are supplied with β-adrenoreceptors and the myoepithelial cells with α-adrenoreceptors (Emmelin and Gjörstrup 1973). In the frog's mucous glands the contractions of the myoepithelium have been shown to be β-mediated (Skoglund and Sjöberg 1977) and in the present experiments the ionic outflow has been shown to be inhibited by β-blockers (cf. Tomlinson and Wood 1976). Since the α-blocking agents had no effect on the ionic outflow there is no reason to assume different receptors for the secretory epithelium of the frog glands.

It might be argued that the simultaneous activation of a large number of glands resulting from suprathreshold stimulation of the sympathetic fibers represents a situation not often occurring under physiological conditions. *In situ* the glands may be reflexly activated by exteroceptive stimuli of various kinds, from light touch to pain stimulation (Engelmann 1872). These types of stimuli usually cause relatively small and shortlasting outflows of secretion (cf. Skoglund and Sjoberg 1977). It was frequently observed, however that the transfer of a frog from the storing basin to a glass beaker caused a large outflow of mucous secretion which might amount to several milliliters. This secretion, which may serve as part of a defense mechanism, could be due to the mechanical skin stimulation while handling the animal but could also—and to a greater extent—be caused by a general excitement of the animal. Whether such a secretion is elicited by impulses in sympathetic nerves or by an increased outflow of neurohormones in the circulation, or both, cannot be decided. But to judge from the large amount of mucus that is secreted during a relatively short time period it might represent a maximal outflow from most of the glands, comparable to that obtained by maximal nerve stimulation.

Our endeavor to check if the relationship between NaCl and water established for the secretion during neurohormonal stimulation *in vivo* (Campbell *et al.* 1967) also holds for the secretion induced by nerve stimulation in our preparation, prompted us to try to measure volume changes in the test compartment. However as described above, interference from tension changes in the skin prevented any reliable estimation of the volume changes. It is therefore not possible to determine if the ionic outflow is always accompanied by water in the same proportion as under physiological conditions. Nor do we know if the production of mucus is kept up at normal rate during the intense activation of the gland during long periods of nerve stimulation.

The possible mechanisms behind the tension changes induced by sympathetic nerve stimulation have been discussed above.

This work was supported by the Swedish Medical Research Council (Project No 04X-4490) and by the funds of Karolinska Institutet

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In Vivo Studies of Individual Mucous Glands in the Frog

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Received 21 February 1977

Abstract

Skoglund, C. R. and E. Sjöberg. *In vivo studies of individual mucous glands in the frog.* Acta physiol. scand. 1977 *100* 471–484

Individual mucous glands in the toe web were studied in curarized decerebrate frogs using vital microscopy in combination with still or motion photomicrography. By changing the focus position to different levels various structures in the gland could be identified and their changes during glandular activation studied. The first visible effect of nerve stimulation was a contraction of the myoepithelium and probably also structural changes of the secretory epithelium resulting in a narrowing of the glandular lumen. Following this, the tricuspid valve opened and secretion was ejected. The latency and time course of the contractile response to nerve stimulation were determined and the influence of the number of stimuli on the duration of the contraction and relaxation phases was analyzed. Comparisons were made with reflex activation of the gland as well as with neurohormonal stimulation. The myoepithelial contraction was found to be under adrenergic control. Of the smooth-muscle stimulants tested only Substance P induced contractions. The time course of the ionic outflow from the toe web was determined by conductance measurements of the fluid surrounding the web and compared with the visually observed phenomena. The initial outflow was concomitant with the phasic myoepithelial contraction but continued secretion could also be observed and recorded from glands kept in a steady state of contraction by iterative nerve stimulation. The functions of the toe web glands were found to be critically dependent on a maintained circulation in the surrounding capillary network.

In the preceding paper (Skoglund and Sjöberg 1977) the quantitative ionic outflow from the frog's mucous glands in nerve-skin preparations *in vitro* was studied by means of a method based on conductance measurements (Lang *et al.* 1975). Although small, the calf skin area studied was estimated to contain a few thousand glands. The accumulated effects of a large number of glands were a prerequisite for the quantitative determinations since the minute outflow of individual glands was beyond the sensitivity of the method used. In the main series of experiments the relation between ionic outflow and nerve stimulation was studied under varying conditions. During the course of the investigation several issues arose, e.g. the different roles of the myoepithelium and the secretory epithelium in the various phases of the secretion process. It soon became obvious that in order to illuminate these problems more information was needed about the behavior of the individual glands.

However the calf skin preparation proved to be less suitable for such studies and we

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g. 1 Cross section of frog toe web in Crossman aining, showing two mucous glands in the connective tissue between the two darkly stained epithelial yers. Duct of left gland projects through the epithelium to the surface.

rtion. The large acinolated secretory epithelial cells are seen projecting into the lumen the gland. They are surrounded by flat elongated myoepithelial cells appearing as a rk layer in the picture. Between the myoepithelium and the gland's connective tissue psule nerve terminals are visible (at arrow). As shown by Sjöberg and Flock (1976) these rminals are located at a distance of 0.5 µm from the myoepithelial cells. No terminals ere found in contact with the secretory epithelium. The gland duct passes through the ithelial cell layer of the epidermis and ends in an opening at the surface in the shape of a icuspid valve formed within a single cell (see inset in Fig. 2).

The broken lines in Fig. 2, at the upper pole and the equatorial area of the gland respectively indicate the approximate levels of the horizontal histological sections A and C in Fig. 3. In A the outer contour of the neck is seen in the center of a narrow lumen surrounded y secretory cells with darkly stained nuclei. In C at the equatorial level, the lumen is larger nd filled with an opaque content. When viewing the gland in the microscope at the same agnification the image varies with the focal position. In B the focus is set at the upper pole nd a picture similar to the cross section in A is obtained. Thus, the small bright glandular pening in the center is visible and the neck formation and the secretory cells can be identified. n D the focus is shifted 30 µm deeper towards the equatorial level and here the picture esembles that in C. The glandular opening is now off focus and the outer circumference ppears larger. To be able to assess changes in the shape and size of the gland the focus as obviously to be kept at a constant level. The inner and outer contours of the glandular



ig. 2. Cross section of mucous gland. Composite of lection micrographs. Broken lines through upper ole (A–B) and equatorial region (C–D) indicate he levels of the histological sections and photomicrographs A, B, C, D in Fig. 3 respectively.

therefore turned to another preparation, the toe web. In 1840 Ascherson reported that the glands in the frog's toe web could be studied *in vivo* and described changes in the size and shape of the glands occurring spontaneously or as results of chemical stimulation. Some thirty years later Engelmann (1872)—expressing his surprise that none of the many researchers studying frog skin in the meantime had taken up Ascherson's preparation—published an extensive study of the behavior of the mucous glands in the frog's toe web using various modes of activation, including nerve stimulation. More than a hundred years have now elapsed since his study was published but as far as we are aware—to our own surprise!—no one has since then utilized this preparation.

With modern microscopic technique it was easy to confirm and extend Engelmann's observations; cinephotomicrography has also made it possible to follow the time courses of the visible changes in the gland structures.

The investigation reported below is mainly concerned with effects of nerve stimulation and reflex activation but some pharmacological results will be presented as well. An attempt has also been made to correlate the visually observed changes with the time course of the ionic outflow from the toe web.

Methods

The *in vivo* studies of the toe web were performed on pithed, curarized frogs of the species *Rana temporaria* and *Rana esculenta* as well as *Xenopus laevis*. The Reichert Biovert microscope used, which is of the inverted type, allows of keeping the whole frog on the gliding stage plate with ample room for various manoeuvres between the preparation and the condenser, such as electrode application and administration of drugs. The web between two of the toes was spread out and placed on a cover glass and kept moist by intermittent application of Ringer solution. The sciatic nerve on either side was dissected free and placed on stimulating electrodes.

Stimuli of 3 ms duration and minimum 5 V from a Grass S4 stimulator were applied either single or repeated at varying intervals. Planachromatic optics or interference contrast according to Nomarski was used for observations and photography. The 16 mm Bolex Paillard cine camera was equipped for marking of the stimulation periods on the film.

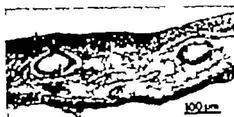
In some experiments the time course of the ionic outflow from the web was recorded by placing a conductance probe close to the surface of the web which was submerged in a shallow bowl with distilled water. This procedure is a modification of a method previously used for quantitative determinations of the ionic outflow in excised nerve–skin preparations and described in detail in previous papers to which the reader is referred (Lang et al. 1975, Skoglund and Sjöberg 1977). The conductance changes were recorded on one channel of a Grass polygraph, another channel being used for marking of the nerve stimuli. In a few instances mucous glands in excised nictitating membranes kept in Ringer solution were also studied.

Results

Observations on individual glands

The mucous glands in the frog's toe web are located in the connective tissue between the ventral and dorsal epithelial surface layers, as illustrated in Fig. 1. The toe web has a thickness of about 200 μm and the gland diameters vary from about 50 to 150 μm. The glands, to the number of 30–50 per square mm, open at random either at the dorsal or the ventral surface of the web.

Fig. 2 is a composite of several electron micrographs showing a mucous gland in cross



ig. 1 Cross section of frog ... in Crosssection ... showing ... mucous glands in the con- ... between the ... darkly stained epithelial ... Duct of left gland projects through the epithe- ... to the surface.

... The large vacuolated secretory epithelial cells are seen projecting into the lumen of the gland. They are surrounded by flat elongated myoepithelial cells appearing as a ... layer in the picture. Between the myoepithelium and the gland's connective tissue ... nerve terminals are visible (at arrow). As shown by Sjöberg and Flock (1976) these ... are located at a distance of 0.5 µm from the myoepithelial cells. No terminals ... found in contact with the secretory epithelium. The gland duct passes through the ... cell layer of the epidermis and ends in an opening at the surface in the shape of a ... wnapsid ... formed within a single cell (see inset in Fig. 2).

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... 2 Cross section of mucous gland. Composite of ... micrographs. Broken lines through upper ... (*A*–*B*) and equatorial region (*C*–*D*) indicate ... levels of the histological sections and photo- ... micrographs *A*, *B* and *C*, *D* in Fig. 3 respectively.



Fig 3 *A* histological section through upper pole of mucous gland and *B* in vivo photomicrograph with focus set at same level (marked *A B* in Fig. 2). *C* and *D* corresponding histological section and photomicrograph respectively at equatorial level (*C D* in Fig 2) See text.

epithelium are fairly clear in this picture The quality of the microscopic picture varies somewhat with the transparence of the toe web which may change depending on the degree of pigmentation and amount of mucus on the skin surface.

In one series of experiments we studied the glands of the nictitating membrane which is even more transparent than the toe web Thus, in Fig. 4 *A* showing a relaxed mucous gland, one can clearly see the structural arrangement of the secretory epithelial cells of varying sizes, similar to the formations appearing in Fig. 2. The darker strips at the outer contour of the epithelium might correspond to the myoepithelial layer In the large lumen some dark, rounded structures appear By shifting the focus these were revealed as being projections of epithelial cells at the bottom of the gland. Sometimes, however organized material in the secretion may give similar pictures. Fig. 4 *B* shows the same gland maximally contracted after topical application of adrenaline the epithelial cells are pressed inwards and against each other by the intense contraction of the myoepithelium so that the lumen is no longer visible

As already noticed by Ascherson (1840) "spontaneous" changes in the shape of the glands may be observed These are probably due to inherent variations in the sympathetic tone since they disappear after denervation (*cf* Engelmann 1872) Fig. 5 illustrates successive

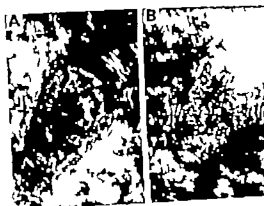


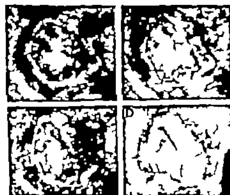
Fig. 4 *In vivo* photos of mucous gland in nictitating membrane. *A* relaxed state *B* maximal contraction after adrenaline administration. See text.



5 Spontaneous contraction of toe web mucous gland. *A* relaxed state; *B* and *C*, successive stages of action occurring within 3–5 s.

s of such a spontaneous contraction, with a symmetrical reduction of the gland size ded with a swelling of the glandular epithelium. This contraction was sustained for about before relaxation set in. As pointed out by Engelmann, and verified by us, structural ges of the glandular epithelial cells may occur simultaneously with the contraction, a detailed analysis of these phenomena has not been performed in this series of experiments.

or a more precise study of the contraction and relaxation properties of the myoepithelium, ulation was applied to the sympathetic fibers in the skin nerve. Fig. 6 *A* shows a gland in normal resting state before stimulation. Application of a single suprathreshold nerve pulse (*B*) results, after a latency of 1.5 s, in a change in the configuration of a sector of outer circumference of the gland as well as of the inner contour of the glandular epithe. Apparently only a limited number of myoepithelial cells are being activated at this e. When the stimulus is repeated before relaxation has set in (*C*) a summation of the tractile effects in this sector occurs. With yet another stimulus (*D*) the contraction increases further and also involves other parts of the myoepithelial network. Additional uli at appropriate intervals induce a further stepwise increase in contraction up to a imum comparable to that in Fig. 5 *C*. The time needed to attain full contraction varies



. 6 Successive stages of contraction of toe web ous gland induced by single nerve stimuli. *A*, mal resting state; *B*–*D*, summation of contraction repeated stimulation. See text.



Fig. 7 Capillary network surrounding toe web mucous glar

somewhat with the frequency of stimulation but at 10 Hz it amounts to about 2–3 s. The fact that only a certain sector of the myoepithelial network participates in the contraction in spite of supramaximal nerve stimulation, as in this case, might be due to some blocking effect occurring at the terminals or more proximally in the ramifications of the nerve supplying the gland. In other glands a symmetrical contraction is initiated by the nerve stimulation even at its onset. Both types of behavior occur on reflex activation as well (*cf* below).

When the stimulation is discontinued the contraction is maintained for a certain period of time that is related to the duration of the previous stimulation. On application of only just the minimum number of stimuli needed to attain full contraction the relaxation seems to start very soon after cessation of stimulation. Following longer stimulation periods of 10 to 20 s and even up to 30 s, some 15–20 s may have to pass before the first sign of relaxation can be observed. Under such conditions it is very likely that an excess amount of transmitter has been liberated which has the same sustaining effect on the contraction as a topical application of an "overdose" of adrenaline which maintains the contraction until the active substance has been rinsed out. The time needed for complete relaxation, *i.e.* back to resting state, also varies with the degree of contraction, from some 10 s up to several minutes. Thus in an experiment in which nerve stimulation was applied during 10 s at 10 Hz full relaxation was not attained until after 2 min, and in another case with a stimulation period of 30 s at 10 Hz the gland was still in a state of moderate contraction after 2 min, full relaxation being reached only after 5 min. It should be emphasized, however, that the myoepithelium invariably responds to a new stimulus even before full relaxation has been attained. The variations in relaxation times that may be observed from one experiment to another are most likely due to the preparations being in more or less good condition.

In a well circulated preparation one and the same gland may contract an infinite number of times provided that a resting period of at least 5 min is allowed between the stimulations. This is the case also after longlasting stimulation periods. One gland was thus followed over an observation period of more than an hour. During that time the gland was seen to contract on renewed stimulation 10 times without showing any obvious sign of fatigue even though the stimulation periods had been as long as 30–60 s. On the other hand, when a new contraction was induced before full relaxation had been attained it was observed that even after 3–5 contractions a refractory state set in during which no response whatever or only a weak contraction resulted.



. 8 Droplet formation at mucous gland opening. See text.

Observations on a great number of preparations revealed that the myoepithelial contrac
was very dependent on a good circulation. Normally the blood flow in the capillary net
of the toe web is vivid. The contact between the capillaries and the gland is very close-
a capillary vessel is seen to form a circlet around the gland, as illustrated in Fig. 7
ling of the frog may cause a temporary slowing of the blood flow but usually the circu-
tion returns to normal within half an hour When the capillary flow is slowed down as
result of circulatory failure, e.g. in deteriorating decerebrate preparations, the responses
nerve stimulation become weaker and finally some glands cease to respond.

These effects were studied more systematically in a series of experiments in which the
culation in the toe web was abruptly stopped by tying the femoral artery When testing
e myoepithelial responses to nerve stimulation at 5 min intervals many glands were found
become inexcitable after 25–30 min, but occasionally glands were observed to respond
er periods up to 2 h. The variations in sensitivity to anoxia may be accounted for by
fferences in the initial state of the preparation. It was also evident that during long-term
imulation the myoepithelial response was more easily fatigued when the circulation was
locked. This unresponsiveness is most likely due to a failure of the nerve–muscle transmis-
on since in such cases the myoepithelium was found to react to electrical stimulation
pplied directly to the gland.

The excitability may vary in different glands even in fresh preparations with normal circu-
ation. Thus out of five glands observed simultaneously in the field of view there is usually
ne that gives no response to nerve stimulation. The four reacting glands often differ
markedly in excitability some of them requiring a longer train of stimuli to attain full
contraction. Longlasting observations indicate that the differences in excitability do not
represent a permanent state some previously refractory glands may again respond whereas
others become unresponsive. This phenomenon which is seen also during reflex activation
may indicate a basic mechanism implying a rotation of activity

When focusing on the surface at high magnification the characteristic Y-shaped opening
formed by the tricuspid valve can easily be identified. During stimulation the valve will
open more or less completely An analysis of the temporal sequences on the cine film reveals
that the opening occurs only slightly later than the myoepithelial contraction, apparently
as a result of the increased intraluminal pressure. Powerful contractions not followed by any

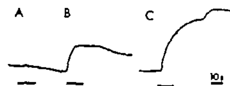


Fig. 9 Conductance records obtained with probe close to toe web immersed in distilled water during nerve stimulation of various strengths. *A*, 3 V no response, *B* 3.5 V sub maximal, and *C*, 5 V maximal response.

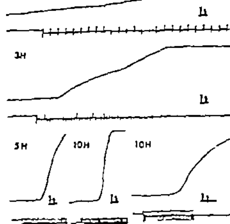
visible change in valve configuration may often be observed this may be a sign of insufficient production of secretion.

Attempts to observe the outflow of secretion were not always successful due to several factors. A more fluid secretion may not form a droplet but may be dissolved in the fluid layer covering the web. On some occasions something like a small jet may be seen coming out of one corner of the triangular opening which may not have fully widened This represents apparently a less mucous secretion than that forming a droplet that stays attached to the opening for some time before spreading out as is typically illustrated in Fig. 8 from an experiment in which the formation of secretion could be followed during repeated stimulations In *A* it is possible to see the contours of the closed tricuspid valve before stimulation and in *B* the round droplet covering it after stimulation After a while the droplet floats out into the medium the valve is seen again (*C*) and the process can be repeated. In this experiment the gland was stimulated during various stimulation periods and at different intervals. When the gland was stimulated for a brief period of 5 s a new droplet was always formed on repeated stimulation some 10 s afterwards However after longer periods of continuous stimulation, of 20–30 s, there was no noticeable outflow of secretion unless a resting period of a few minutes was allowed

Recordings of the ionic efflux from the toe web

To be able to compare the results from the observations of the individual glands in the toe web with the previous studies of the calf skin preparation *in vitro* an attempt was made to modify the method of conductance measurements so as to suit the *in vivo* preparation. The method used for quantitative determinations of the ionic outflow from the mucous glands in the calf skin preparation *in vitro* (Lang *et al* 1975) implies that the ionic efflux from a given skin area is collected in a small compartment containing a known volume of distilled water whose conductance change after gland activation is a measure of the ionic outflow In the *in vivo* experiments on the toe web it proved difficult to arrange such a collecting chamber without interfering with the circulation and innervation of the web. Instead, the probe was simply placed against the web between two toes and the whole foot was immersed in a shallow bowl filled with distilled water It is obvious that this arrangement does not allow a quantitative determination of the outflow of ions resulting from a certain period of stimulation, but it permits a recording of the temporal course of events and can also give information about the relative intensities of the effluxes under varying stimulus conditions.

As appears from Fig. 9 it is actually possible to record conductance changes analogous to those achieved when using the arrangement with a small chamber The probe measures the conductance change occurring at its gap about 0.5 mm above the web surface. The con-



[Fig.] 10 Effect of stimulus frequency on latency of [ionic] outflow from toe web. See text. 1 Hz—8.0 s, [3 Hz]—2.4 s, 5 Hz—2.0 s, 10 Hz—1.8 s. Two expts, [note] time scales.

[conduc]tivity increases as long as the flow of ions into this gap is faster than the disappearance [of i]ons due to diffusion into the surrounding medium. Since this diffusion is comparatively [slow w]hen no stirring occurs, the rising phase of the curve will give a fairly good estimate [of] the rate of the ionic efflux. When the outflow ceases the curve levels out into a plateau [in] the same way as in a closed compartment. However this level will not be maintained, as [it is] after equilibration in a small compartment, but will slowly decrease due to the diffu[sion] into the large volume of the bowl.

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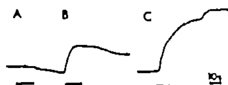


Fig. 9 Conductance records obtained with probe close to toe web immersed in distilled water during nerve stimulation of various strengths. *A*, 3 V no response; *B* 3.5 V submaximal, and *C*, 5 V maximal response.

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Attempts to observe the outflow of secretion were not always successful, due to several factors. A more fluid secretion may not form a droplet but may be dissolved in the fluid layer covering the web On some occasions something like a small jet may be seen coming out of one corner of the triangular opening which may not have fully widened. This represents apparently a less mucous secretion than that forming a droplet that stays attached to the opening for some time before spreading out as is typically illustrated in Fig. 8 from an experiment in which the formation of secretion could be followed during repeated stimulations In *A* it is possible to see the contours of the closed tricuspid valve before stimulation and in *B* the round droplet covering it after stimulation After a while the droplet floats out into the medium the valve is seen again (*C*) and the process can be repeated. In this experiment the gland was stimulated during various stimulation periods and at different intervals. When the gland was stimulated for a brief period of 5 s a new droplet was always formed on repeated stimulation some 10 s afterwards. However after longer periods of continuous stimulation, of 20–30 s, there was no noticeable outflow of secretion unless a resting period of a few minutes was allowed.

Recordings of the ionic efflux from the toe web

To be able to compare the results from the observations of the individual glands in the toe web with the previous studies of the calf skin preparation *in vitro* an attempt was made to modify the method of conductance measurements so as to suit the *in vivo* preparation. The method used for quantitative determinations of the ionic outflow from the mucous glands in the calf skin preparation *in vitro* (Lang *et al* 1975) implies that the ionic efflux from a given skin area is collected in a small compartment containing a known volume of distilled water whose conductance change after gland activation is a measure of the ionic outflow In the *in vivo* experiments on the toe web it proved difficult to arrange such a collecting chamber without interfering with the circulation and innervation of the web. Instead, the probe was simply placed against the web between two toes and the whole foot was immersed in a shallow bowl filled with distilled water It is obvious that this arrangement does not allow a quantitative determination of the outflow of ions resulting from a certain period of stimulation, but it permits a recording of the temporal course of events and can also give information about the relative intensities of the effluxes under varying stimulus conditions.

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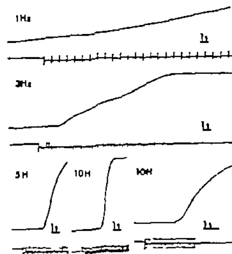


Fig. 10. Effect of stimulus frequency on latency of ionic outflow from toe web. See text. 1 Hz—8.0 s, 3 Hz—2.4 s, 5 Hz—2.0 s, 10 Hz—1.8 s. Two expts; different time scales.

ductivity increases as long as the flow of ions into this gap is faster than the disappearance of ions due to diffusion into the surrounding medium. Since this diffusion is comparatively slow when no stirring occurs, the rising phase of the curve will give a fairly good estimate of the rate of the ionic efflux. When the outflow ceases the curve levels out into a plateau in the same way as in a closed compartment. However this level will not be maintained, as occurs after equilibration in a small compartment, but will slowly decrease due to the diffusion into the large volume of the bowl.

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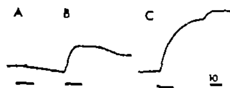


Fig. 9 Conductance records obtained with probe close to toe web immersed in distilled water during nerve stimulation of various strengths. *A* 3 V no response, *B*, 3.5 V submaximal, and *C*, 5 V maximal response.

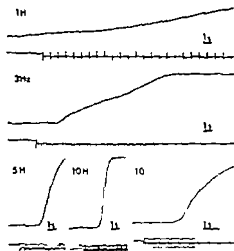
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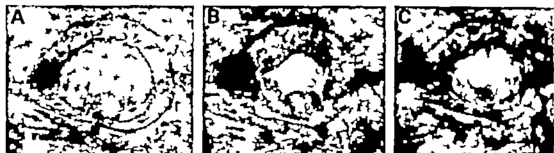


Fig. 11 Contraction of mucous gland in toe web of right foot induced by pinching of left foot. *A*, initial resting state *B*, maximal contraction *C*, full relaxation after 5 min.

Cinephotomicrographic recordings of the individual glands showed that the myoepithelial contraction started about 1.5 s after stimulation and that the opening of the orifice resulting from the increased pressure in the gland occurred within the next 0 5 s. With the type of probe used (*cf* Methods) a certain delay in the recording has to be taken into account; this is due to the diffusion time needed before the ions reach the gap of the probe 0.5 mm above the surface. The actual outlet of ions from the glandular orifice may thus occur some hundred milliseconds earlier than the recorded conductance change. The similarity of the latency values found for the visually observed phenomena of glandular activation and the ionic outflow supports the conclusion drawn from the *in vitro* experiments that the initial ionic outflow is a result of the glandular contractions squeezing out the preformed secretion.

However in the preparation *in vitro* a continuous ionic outflow was also recorded during the later phases of the stimulation periods lasting up to one and occasionally two minutes. It was concluded that this was a secretion caused by nervous activation of the glandular epithelium, which occurred independently of the myoepithelial contractions. Such longlasting outflows could also be recorded from the toe web during prolonged stimulation. In this preparation it is possible to check the contractility state of the glands after long-term stimulation, and all glands observed proved to be in a steady state of maximal contraction. An expulsion of secretion due to a phasic myoepithelial contraction is therefore not likely to occur during the later phases of prolonged stimulation. In agreement with this is also the observation frequently made in the toe web experiments that there is a steady flow of secretion from openings in maximally contracted glands during repetitive nerve stimulation.

Reflex activation

As described by Engelmann (1872) various exteroceptive stimuli are effective in eliciting reflex responses from the mucous glands. We have been able to confirm most of his findings. In agreement with Engelmann we found that the threshold for touch stimulation is very low Receptive areas are found all over the body surface, but to obtain responses from the toe web glands in the hind legs stimulation of the feet seems to be most effective. Even slight mechanical stimulation of the whole preparation caused by movements of the gliding plate of the microscope was found to elicit contractions of the glands, and sometimes a strong sound was sufficient.

A slight touch stimulus applied to the contralateral foot may evoke a more or less full

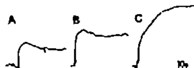


Fig. 12. Conductance records obtained with probe at toe web surface during reflex activation of mucous gland induced by exteroceptive stimulation of contralateral foot. *A*, slight touch, *B*, repeated touch stimulation, *C*, strong pinching. See text.

contraction of the gland under study which indicates that a train of efferent sympathetic nerve impulses must have been initiated by the exteroceptive stimulus. The relaxation sets in very soon, however and the responses appear to be very similar to the "spontaneous" contractions which may sometimes be caused by uncontrollable mechanical stimuli. Repeated touch stimuli as well as a sustained hard pressure on the skin involving also pain fibers will evoke more longlasting contractions. This is illustrated in Fig. 11 Record *A* shows a gland in its initial resting state and *B* the maximal contraction obtained by a strong pinch applied to the contralateral foot by a pair of forceps. The relaxation was slow so that the fully relaxed state in *C* was not attained until after 5 min.

Summation phenomena in response to repetitive stimulation could also be demonstrated by conductance recordings. The response in Fig. 12 *A* is the result of a single touch stimulus applied to the skin of the contralateral foot and *B* the larger outflow after repeating the same stimulus twice. *C* shows the much greater response obtained by stronger pinching activating afferent pain fibers as well. A few experiments were also performed using electrical stimulation of afferent nerves on the contralateral side. Reflex responses from the glands were obtained even at stimulus strengths sufficient to activate afferent fibers of large diameter apparently the same as were engaged during touch stimulation.

When observing some five glands in the field of view there is often one which does not respond in analogy with the findings in the nerve stimulation experiments. In reflex experiments it is also a characteristic feature that a gland that does respond may do so only to some 4–5 stimuli in succession applied to one and the same skin area. This is obviously due to a central habituation process and may be interpreted as part of a mechanism in a rotation of activity as suggested above. As in the experiments with direct stimulation an initially non-responding gland may be seen to contract later on during the period of observation.

Effects of drugs

In the *in vitro* experiments on the calf skin preparation the effects of certain drugs on the mucus outflow were also studied. To be able to establish the role of the myoepithelial contractions in producing the recorded effects the same drugs were applied to the toe web and the myoepithelial responses were observed. Before application of the drugs the contractility of the myoepithelium was always tested by means of reflex or nerve stimulation.

Application of a solution containing 10 μg/ml or more of adrenaline or noradrenaline was found to evoke a complete and sustained contraction of all glands observed. Acetylcholine had no effect whatever

Administration of 40 μg/ml of the β-adrenergic stimulating agent isoproterenol resulted in a glandular contraction similar to that elicited by the catecholamines. The α-adrenergic blocking agent phentolamine did not diminish the responsiveness of the glands to adrenaline,

whereas the β-blocker propranolol in a concentration of 10 μg/ml completely abolished any adrenaline-induced effects.

Dihydroergotamine, which is known to inhibit myoepithelial contractility in higher developed glands (Thulin 1975) was ineffective in blocking the response to catecholamines in the present experiments. Bradykinin physalemin and oxytocin, compounds which stimulate smooth-muscle contractions, were tested but caused no visible contraction of the glands.

Substance P which induces an ionic outflow from the frog skin on topical application (Skoglund and Sjöberg 1977) and which is assumed to play a role in mammalian exocrine gland function, gave a maximal sustained contraction of the mucous glands in a concentration of 0 1 μg/ml

Discussion

Engelmann s (1872) observations on the mucous glands are quite remarkable in view of the microscopes available at his time he described the glandular structures in great detail and studied the reactions of the glands under varying stimulus conditions. The main difference between his experiments and ours is our access to photomicrographic techniques providing documentary evidence for our observations and also enabling us to study the time courses of the events It has thus been possible to get information about the latencies and durations of the myoepithelial contraction and relaxation phases during nerve stimulation.

Very few data seem to be available on the properties of this special type of smooth musculature. The myoepithelial cells in the mammalian salivary gland were studied by Thulin (1975) by recording the pressure changes induced in the parotic duct the latency between the stimulus application and the myoepithelial response was found to be less than one second which corresponds well to the values found in our experiments. Thulin gave no values for the time to peak pressure but from the steep rise of the pressure curves it can be estimated to be in the range of 2–3 s i.e about the same range as was observed for full contraction of the frog myoepithelium. But there are also differences between the muscular tissues of the two types of glands. Thus the transmitter activation of the myoepithelium in the salivary gland is mediated through α-adrenoreceptors whereas in the frog gland the myoepithelium is controlled by β-adrenoreceptors. Furthermore the myoepithelium in the mammalian gland is also under parasympathetic control

Various smooth-muscle stimulants such as bradykinin and physalemin have been shown to activate myoepithelial cells in mammals (Thulin 1976) but on frog myoepithelium these substances had no visible effect. Substance P was found to elicit glandular contraction and this might be the mechanism behind the ionic outflow caused by this drug in the *in vitro* preparation. The powerful contractions induced by catecholamines were to be expected in view of their probable roles as natural transmitters. Adrenaline and noradrenaline might also exert a direct action on the glandular secretory epithelium judging from the longlasting outflows produced by these agents in the *in vitro* preparation. We have not made any systematic attempts to study these effects in the toe web glands. However it should be possible by using high magnification and Nomarski optics to determine if structural changes of the glandular epithelium occur

The myoepithelium of the toe web glands is fairly susceptible to an occlusion of the cir

-lation. Under the experimental conditions applied in our experiments the maximum time period over which contractions could still be observed after the femoral arteries had been clamped was only about one hour. This sensitivity to oxygen lack seems to apply also to the outflow of ions which is considerably reduced within the same period. Thus the behavior of the toe web glands differs markedly from those of the calf skin in which the ionic outflow can be elicited by nerve stimulation for hours and even days in an *in vitro* preparation. It is however possible that the difference is less an expression of inherent properties of the glands proper than of their environment. The calf skin glands are surrounded by a tissue that may have a greater capacity to accumulate oxygen than has the thinner toe web. From early investigations (Hevesy *et al.* 1935) it is also known that the permeability of the toe web to water is very low as compared to that of the leg skin, and this may also apply to the oxygen transport.

In the preceding paper describing *in vitro* experiments it was assumed that the initial outflow during stimulation is a result of the myoepithelial contractions squeezing out the preformed secretion. In the present study the contraction of the gland and the subsequent formation of droplets could be directly observed in the initial stage of nerve stimulation. However, an outflow could be recorded—as well as observed—also in the later stages of stimulation in which practically all glands were in a steady state of contraction. This finding supports the concept of a secretion mechanism independent of the phasic myoepithelial contractions.

It has not been possible to obtain quantitative and qualitative data on the ionic outflow from individual glands. The droplet formed at the gland opening has a diameter of only about 10 μm and even if it can be handled by micropipette technique it is obvious that a chemical analysis of its content would imply certain difficulties. Seldin and Houbiko (1966) have calculated the volumes of droplets from mucous glands to be about 1–4 pl. It is not clear whether the composition of the secretion is the same during intense nerve stimulation as during physiological activation. It is possible that during artificial stimulation the production of mucous constituents does not keep pace with the release of ions and water.

When observing a number of glands in the field of view it was quite striking that all glands did not respond to a maximal nerve stimulation; a previously non-reacting gland might however respond again later on. The same phenomenon could be observed on reflex activation as well. Several reasons can be suggested for this. It might be caused by accidental blockade of the transmission in fine nerve branches supplying a certain gland, or it might be due to a purposeful mechanism protecting the gland against an overstimulation similar to that demonstrated for neuromuscular transmission (Krnjević and Miledi 1958). However our observation periods were not long enough to permit safe conclusions to be drawn about a possible rotation of activity serving a useful purpose under physiological conditions.

The prompt reflex responses obtained from individual glands even to very light exteroceptive stimuli are striking. The toe web gland seems to be a suitable effector organ in studies of reflex transmission from the somatic to the sympathetic nervous system. The organization of the receptor fields has been only briefly analyzed in this investigation and the preparation offers also good possibilities to study the phenomena of habituation on a single-unit level.

This work was supported by the Swedish Medical Research Council (Project no 04X-4490) and by the funds of Karolinska Institutet

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Endurance of Muscle Contraction under Hypnosis

By

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The influence of hypnotic suggestion on muscular performance is a question of debate that has been examined in only a few scientific experiments. Eysenck (1943) suggested that performance in general, including muscular strength and endurance, is increased under hypnotic suggestion. Roush (1951) reported a very significant increase in muscle force under hypnosis but a less significant increase in endurance. Ikai and Steinhaus (1961) showed that muscle strength could be increased by 20 to 30 per cent in hypnosis, but they observed no effect of hypnosis on a competitive weight lifter who could voluntarily maximize his performance. In a recent study Moškin and Poberezhskaya (1976) claimed that hypnotic suggestion increases the duration of maintenance of static effort by twice that in the control experiment.

The purpose of this study was to find out whether the mechanism of muscle contraction is more resistant to fatigue under hypnosis than during voluntary effort. Three female students aged 20 to 22 years were used as subjects. They volunteered for the experiments and were known on the basis of prior experience to fall into deep hypnosis. Isometric knee extension force was recorded with a strain gage coupled to an inkwriter as described before (Komi and Viitasalo 1976). Simultaneously the electromyogram was recorded with surface electrodes from the belly of *m. rectus femoris*. Maximum force was first recorded twice for each leg and 60 per cent of maximum force levels were computed (Table I) The times of maintenance of a 60 per cent force level were then recorded together with EMG To ensure the maintenance of the required force level the inkwriter was placed so that the subject could easily see the force reading After sufficient recovery of several hours the subjects were deeply hypnotized and the same measurements were repeated using the opposite leg. In both parts of the experiment the subjects were repeatedly instructed to maintain the required force level In voluntary contractions the instruction was simply to maintain the required force, whereas under hypnosis the subjects were repeatedly told that the force reading remained the same by itself

In all experiments performed under hypnosis the endurance times were slightly longer than in the control measurements, but the differences amounted to only from 7 to 22 per cent (Table I). No clear differences were seen in the electromyogram recordings between the two experimental conditions. In spite of the repeated telling that the force remained equal in the hypnosis experiments, the force levels finally dropped sharply (Fig. 1). When awakened from hypnosis, the subjects reported that the muscle tension had remained at the said level

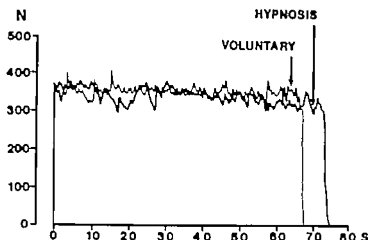


Fig. 1 Maintenance of 60 isometric knee extension by subject no. 2 during voluntary (thin line) and hypnotically induced line) contraction.

with no effort of their own and that the force finally dropped by itself without their control

In another experiment performed under hypnosis the same subjects were given a weight that represented 30 per cent of maximal arm abduction force and told that the arm remains extended horizontally in an abducted position with the weights loaded at the wrist. The subjects kept the weights in this position on the average for 132 s (range 127 to 160). This time is in agreement with endurance times for voluntary contraction, since at a 30 per cent level this should be slightly over 2 min (Rohmert 1960)

Our subjects were used to fall into hypnosis and during the experiments they reached the state of deep hypnosis. This was evidenced by catatonic maintenance of unloaded limb positions, hypnotically induced analgesia and the subjects own ratings of their hypnotic states. Nevertheless, as compared to voluntary contractions the increases observed in the endurance times under hypnosis were small. Our results are in agreement with those published earlier by Eysenck (1943) and Roush (1951) but Moikin and Poberezshkaya (1976) reported much greater increases in endurance of muscle contraction under hypnosis. A critical factor that may explain this difference is the achievement of full motivation for maximal performance in voluntary contractions. If this level is not achieved the results are likely to improve considerably under hypnosis (Ikai and Steinhaus 1961)

TABLE I Endurance times in voluntary (V) d hypnotically induced (H) isometric contraction of the knee extensor muscles. The level of contractio was 60% of the maximum force measured under respective conditions.

	Subject 1		Subject 2		Subject 3	
	Right	Left	Right	Left	Left	Right
Maximum force I, N	570	650	590	620	520	500
Maximum force II, N	610	680	600	600	600	510
60% force level, N	350	400	360	370	340	300
	V	H	V	H	V	H
Endurance time, sec	58	63	67	72	7[illegible]	[illegible]

Contraction force that represents 60 per cent of the maximum voluntary tension obstructs the blood supply of the contracting muscle (Eklund 1974) leading to fatigue when the energy reserves of the muscle are sufficiently depleted. At this contraction level exhaustion occurs in approximately 1 min (Rohmert 1960). In hypnotic and voluntary contractions the peripheral energy supplying mechanisms appear the same. In the study of Molkin and Poberezhskaya (1976) a large increase in the EMG activity was reported under hypnosis, but no clear difference in the integrated EMG between the two states was observed in the present expts. Thus we conclude that in voluntary and hypnotic states endurance of muscle contraction is regulated by similar energy supplying mechanisms and roughly similar spinal motoneuronal commands which are reflected by approximately the same integrated EMG activities. The subjective experience of muscle contraction, however, is profoundly different in the two states indicating a difference in the brain mechanisms involved.

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Acta physiol scand. 1977 100 488–490

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Increase in Paradoxical Sleep in the Cat after Phentolamine, an Alpha Adrenoceptor Antagonist

By

P T S PUTKONEN and A LEPPÄVUORI

Cerebral noradrenaline (NA) is implicated in the control of paradoxical sleep (PS), but its role is controversial According to Jouvet (1975) NA-ergic neurons would play an active role in the executive mechanism of PS while others (*e g* Stern and Morgane 1974) maintain that PS is inversly related to the availability of NA at synaptic sites. Neuropharmacological expts. in man have often been interpreted in favor of the latter hypothesis (Wyatt *et al.* 1971), which has lead to the suspicion that animal models would not hold for the human brain. A similar controversy is also apparent in the few published reports on α-receptor blockade and PS in cats (Matsumoto and Watanabe 1967) and humans (Oswald *et al.* 1975)

In a series of neuropharmacological expts. designed to approach the control of PS and catecholamine receptors in cats we have found an important increment in PS after injections of an α-blocking agent, phentolamine.

Adult cats with fixed electrodes in sensorimotor and visual cortices (EEG), lateral geniculate bodies (PGO) and nuchal muscles (EMG) were used 16 h records with a paper speed of 5 mm/s were visually analyzed in 1 min pages. The states of vigilance were classified to 5 (or 3) stages according to the criteria proposed by Ursin (1968) (see Table I). At the start of the records 6 cats received either single intraperitoneal injections of 20 mg/kg phentolamine (Regitin®) or 5 ml of NaCl (control)

After phentolamine the cats appeared behaviorally sedated. The eyelids and the nictitating membranes were half closed indicating an intense peripheral α-block. Otherwise no discomfort or untoward side effects were noticeable. The effects on sleep are presented in Fig. 1 and Table I For the whole 16 h records (Table I) the only significant ($P < 0.001$) change after phentolamine is a *ca* 62 increase in PS as compared to control In successive 4 h periods (Fig. 1) an increase in PS is evident already in the first 4 h ($P < 0.05$), reaches its maximum (*ca* 120% above control, $P < 0.01$) between 4 to 8 h and levels off after 12 h.

Phentolamine is a relatively potent and specific α-adrenergic blocking agent of competitive type The increase in PS observed by us is contrary to the results of Matsumoto and Watanabe (1967) who reported a decrease in PS in the cat after dibenamine and phenoxybenzamine, two drugs producing long lasting, non equilibrium type of α-blockade. Their result, however might not be specific to the α-receptor blockade, since it could be only obtained with the largest doses (15 mg/kg) which may also inhibit receptors for 5-HT acetylcholine

Table 1. Mean percentages (± S.D.) of the states of vigilance in 16 h after intraperitoneal injections of 5 ml NaCl (controls) or 20 mg/kg phentolamine. Δ % refers to change in per cent of control. P values according to t-test

	Controls (n = 6)	Phentolamine (n = 6)	Δ %	P
Aroused (A)	13.6 ± 3.7	15.8 ± 4.1	+ 16.2	N.S.
Drowsy (D)	16.8 ± 8.7	10.9 ± 5.[illegible]	− 35.1	N.S.
Awake (A + D)	30.4 ± 5.1	26.7 ± 7.2	12.1	N.S.
Light slow wave sleep (S1)	22.6 ± 8.6	23.5 ± 4.1	4.3	N.S.
Deep slow wave sleep (S2)	33.0 ± 7.9	27.1 ± 5.3	17.9	N.S.
Slow wave sleep (S1 + S2)	55.6 ± 6.5	50.6 ± 5.1	9.0	N.S.
Paradoxical sleep (PS)	14.0 ± 2.2	22.7 ± 3.4	62.1	0.001

and histamine (Nickerson and Collier 1975). Also according to our experience phenoxybenzamine becomes fatally toxic to cats at ca. 20 mg/kg.

On the other hand, Oswald *et al.* (1975) have reported that i.v. infusion of another α-receptor blocker thymoxamine (150 mg/kg) can increase the duration of REM-sleep in young adults. Our results indicate that cats may after all not differ from humans in this respect. Further evidence for the notion that α-blockade generally favors PS can be derived from our unpublished results that phentolamine and other α-antagonists can counteract the PS-reducing effects of clonidine (Leppävuori *et al.* 1976), a claimed central α-receptor agonist.

Coming back to the problem whether NA participates in PS as a predominantly positive or negative agent, our results, however, are not conclusive. Alpha receptor blockade might have even opposite functional consequences depending on the timing and balance of the effects between the postsynaptic receptors mediating effector responses and the presynaptic receptors participating in negative feedback loop controlling neurotransmitter release (Andén et al. 1976). Also interactions between the NA-ergic and 5-HT-ergic systems (Jouvet 1979) may further complicate the picture.

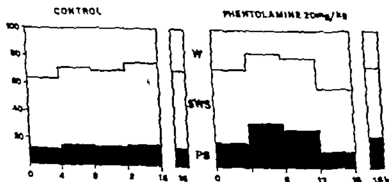


Fig. 1. Percentages of the states of vigilance in successive 4 h epochs, and for the total 16 h records in 6 cats receiving either i.p. NaCl (control) or phentolamine at the beginning of the recording. W = waking, SWS = slow wave sleep, PS = paradoxical sleep

This work was supported by grants from Academy of Finland, National Research Council for medical sciences.

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Observations on Strength Training and Detraining

By

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-o earlier studies (Thorstensson *et al.* 1976 a and b) the effect of 8 weeks of heavy ...ssive strength training were investigated in 22 male students of physical education. ...y the results showed that the training regimen caused a large gain in strength per ...nce and an increase in muscle mass, but only minor changes in muscle f bre charac...ics, enzyme activities and EMG One subject (E.G) continued training after the initial ...od and was reexamined 5 months later along with another subject (A. M.) who had ...een participating in any strength training for the corresponding period of time. This ...r reports results from strength tests, anthropometric measurements and muscle fibre ...res for these two subjects before (Test I) and after (Test II) the initial training period as ...as after the additional 5 months (Test III).

...e methods and test procedures used were described in detail in the papers mentioned ...e. Added to the protocol were measurements of dynamic strength of the quadriceps mus... This was done with an isokinetic dynamometer at different constant angular velocities (see ...rstensson *et al.* 1976 c). The initial progressiv strength training program was common ...he two subjects and consisted mainly of maximal or close to maximal exercises for the ...xtensor muscles, either with weights or as different jumping regimens (for details see ...rstensson *et al.* 1976 a and b). Subj. E. G (age 23 yrs, height 182 cm, weight before ...ing 80.5 kg) continued his strength training according to a similar protocol, but with ...ewhat lower training load. The number of sessions was decreased from 3 to 2 per week, ...lting in a decrease in mean weekly training load in squats from 7 730 kg to 4 320 kg. ...thermore, the different jumping exercises were replaced by strength training exercises ...the upper body e.g. bench press Subj. A. M. (age 4 yrs, height 179 cm, weight before ...ning 69 5 kg), who during the initial 8 weeks performed a mean weekly training dose of ...0 kg in squats, did not take part in any strength training after this period. Apart from ... differences in training, the daily activities of the two subjects were very similar Both ...them were living at the same school and taking the same courses.

The initial 8 weeks of common strength training caused substantial improvements in ...ctional performance tests (Table I), essentially of the same order of magnitude for both ...bjects In Test III Subj E. G showed further increase in 1 RM in squats, whereas the ...rformance decreased in the functional tests omitted from the training schedule. However ...e results were still well above the before training values. For Subj. A. M. 5 months of ...sence from strength training caused a decline in strength performance to values attained

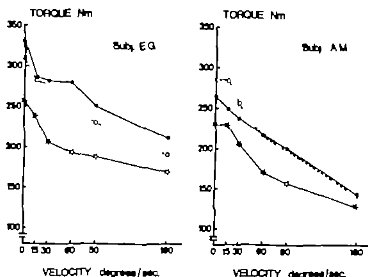


Fig. 1 Peak torque values (Nm) produced during maximal leg extension with different angular velocities (degrees/sec). ☆ before training, ○ after 2 months of strength training and ● after an additional 5 months period of strength training (Subj. E. G) or non-training (Subj A. M), respectively

before the initial training period, except for 1 RM in squats for which the decrease was less marked. Peak torque of the quadriceps muscle demonstrated an average increase of 25% for both subjects at all constant velocities examined (method error 3–4%, Thorstensson 1976) except for the highest speed (180 degrees/s) at which the increase was only about 10% (Fig. 1) In Test III Subj E. G showed further increase in peak torque. This time however the increase was largest at the highest speed Subj A M showed a decrease in peak torque at the low speeds whereas the values at the high velocities were similar before and after the 5 months of non-training (Fig. 1). The explanation for this apparent speed specificity might be derived from common activities not controlled in this study although the specific response of Subj E. G could be caused by the change from heavy weights in the first period to somewhat lighter loads and thus higher speeds during the second training period

TABLE I Results from functional tests, anthropometric measurements and muscle fibre analysis, I before training, II after months of strength training and III after an additional 5 months period of strength training (Subj. E. G) or non-training (Subj. A M), respectively

	Subj E. G			Subj A. M		
	I	II	III	I	II	III
1 RM in squats, kg	140	230	245	125	215	180
Vertical jump, cm	56	71	67	61	64	61
St. broad jump, cm	242	262	258	245	255	242
Total leg force, kp	222	247	230	192	205	188
Body weight, kg	80.5	81.5	85 1	69 5	69 4	70.5
Lean body mass, kg	72.2	75.5	77 6	64.2	65.5	60.6
Total body K, g	174.5	183.3	188.9	155.3	158.8	145 6
Muscle mass, kg	35.5	38.9	40.1	32.5	33.9	28.3
Thigh girth, cm	55 6	56.6	57 1	52.5	51.5	53.0
Calf girth, cm	39 6	40.1	40.6	35 9	36.0	35.3
Upper arm girth, cm	35 4	35 7	38 1	31 1	31 4	31 7
FT %	62	66	67	52	51	57
FT/ST area ratio	1.32	1 49	1.37	1.33	1 69	1.59

milar gains in anthropometric measures, e.g. total muscle mass and thigh girth were d for both subjects in test II (Table I). There were only minor changes in b.wt., indi- g a change in body composition towards less fat tissue (cf Thorstensson et al. 1976 a, b). nthropometric measurements showed a further increase for Subj. E. G in Test III, cating training induced muscle hypertrophy (Table I). The pronounced increase for the er arm could be derived from the addition of arm exercises to the training program. Subj. A. M. demonstrated a gain in b.wt. in Test III In his case, however the eased b.wt. was due to an augmented amount of fat, since lean body mass, total body nd calculated muscle mass were decreased (Table I). Skinfold measurements in the ominal region showed an increase from 10 to 19 mm over the 5 months period (the responding values for Subj E. G were 12 and 11 mm, respectively).

Muscle fibre analyses of needle biopsy samples from the vastus lateralis muscle showed or changes in muscle fibre distribution (% fast twitch fibres, FT) after the first 8 weeks of ning. However the FT to ST (slow twitch) fibre area ratio increased with training for h subjects (Table I) indicating a selective hypertrophy of FT fibres (cf Thorstensson 76). The percentage of FT fibres was unchanged for Subj E. G in Test III, whereas the /ST area ratio was somewhat decreased as compared to Test II. The same trend arding fibre area ratio was evident for Subj. A. M The fibre distribution seemed to be nged towards a higher percentage of FT fibres after 5 months of resting from strength ining. However great precautions have to be taken when evaluating these results con- ering the relatively large method errors involved. The coefficients of variation for % FT d FT/ST area ratio determinations have been reported at 10% and 9%, respectively horstensson 1976).

Comparison of the two subjects in the present study has shown that increases in strength d muscle mass acquired during 2 months of training were essentially lost after 5 months of n-training. It has also indicated the specificity of the training as well as the detraining ponse. In light of the relatively few studies reported concerning the effects of detraining, r results of the present paper might offer some clues for further investigation.

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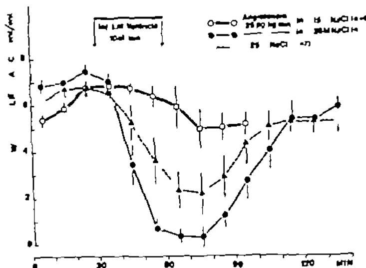
Cerebral Sodium-Angiotensin Interaction Demonstrated with "Subthreshold" Amounts of Angiotensin II

By

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Investigations performed by Fitzsimons and coworkers (*cf* Fitzsimons 1972) imply th activation of the renin-angiotensin system forms a link in a volumetric regulation of wa intake in the rat, and have demonstrated that angiotensin II acts as a very powerf dipsogen when applied intracranially at the hypothalamic level The centrally mediat thirst-eliciting effect of this octapeptide has later been confirmed in several other specie and angiotensin II has been shown to stimulate also the release of antidiuretic hormo (ADH) by an action at the hypothalamic level of the brain (*cf* Severs and Summy-Lo 1975) It still remains doubtful however whether endogenously produced angiotensi affects cerebral control of water balance in other mammalian species than the rat. In t sheep f ex. Abraham *et al* (1975) have found that the intracarotid and intracerebroventricu lar (IVT) dosages of the octapeptide needed to induce drinking are nonphysiologic Abraham *et al* have also measured the cerebrospinal fluid (CSF) angiotensin II concen tration in sheep during maximal stimulation of the renin-angiotensin system (durin severe sodium depletion) In extrapolating their data to previous experiments (Anderss *et al* 1972) they concluded that even the lowest IVT dosage of angiotensin II used to induc drinking and ADH-release in the goat ought to have raised CSF angiotensin concentratio 10–50 times maximum physiological level However angiotensin II markedly strengthe the dipsogenic and ADH releasing effects of elevated CSF [Na] in the goat (*cf* Andersso 1971) Therefore, it was of interest to study whether this kind of central sodium-angiotensi interaction could be demonstrated with an IVT dosage of angiotensin II which accordi to Abraham *et al* possibly falls within the physiological range.

5 female goats (b wt 30–35 kg) were used. The animals were supplied with a platinum-iridium cannulas in the lateral cerebral ventricle where IVT infusions were mad at a rate 10 μl/min. The construction of the cannula-system and the infusion technique hav been described earlier (Åkerlund, Andersson and Olsson 1973) The following IV infusions were made with a minimum interval of 2 days in each animal a) Angiotensin I (Hypertensin, "Ciba") 25 pg/kg min in isotonic (0.15 M) NaCl, b) the same dosage o angiotensin in hypertonic (0.25 M) NaCl and c) hypertonic (0.25 M) NaCl The ADH releasing response to 30 min infusions (judged by the degree of temporary reduction o renal free water clearance (C_{H_2O})) was studied in 3 hydrated goats during fully establishe water diuresis. The dipsogenic response (= amount of water drunk and latency t



...engthening of the antidiuretic response to IVT infusions of hypertonic (0.25 M) NaCl by small, ...gely ineffective amount of angiotensin II. The various infusions were performed at random order ...ted goats. Vertical bars = S.E.

) was determined during 20 min infusions in three of the goats when not hydrated. *release* The IVT infusions of angiotensin in isotonic NaCl made in hydrated (n 6) caused only slight post-infusion reduction in renal C_{H_2O} (Fig. 1 *circles*) ...g that ADH-release in response to these infusions was negligible. In contrast, the ...s of the same amount of angiotensin dissolved in hypertonic NaCl (n = 7) resulted ...st complete inhibition of the water diuresis (renal C_{H_2O} near 0) which outlasted the ... period by about ½ h (Fig. 1 *dots*). The antidiuretic effect of the infusions of merely ...nic NaCl (n 7) was less pronounced than that obtained during the combined ...s (Fig. 1 *triangles*).

... One of the pre-hydrated goats used for studies of ADH-release invariably ... relatively large amount of water (270 ± 23 ml) during the infusions of angiotensin ...rtonic NaCl (4), but payed no attention to the water during the other two kinds ...sion. Therefore, the d psogenic effect of the various IVT infusions was tested also in ...nhydrated goat. No drinking occurred during 6 out of 7 infusions of angiotensin in ...c NaCl. In one expt. 110 ml of water was taken towards the end of the infusion. Both ...er two kinds of infusion caused drinking. However as seen in the following table the ...enic response to angiotensin in hypertonic NaCl was considerably greater than that ...rely the hypertonic NaCl

...T Infusion	Angiotensin/0.25 M NaCl (n = 8)	0.25 M NaCl (= 8)
...ter drunk ...Mean ± S.E.)	653 ± 140 ml	280 ± 116 ml
...tency (Mean)	8 min	12 min

On the basis of experiments demonstrating that the dipsogenic and ADH releasing effects of angiotensin II are highly dependent upon the prevailing CSF [Na] it has been suggested that the octapeptide may influence periventricular Na transport in a manner which facilitates the excitation of sodium-sensitive juxtaventricular receptors involved in the cerebral control of water balance (Andersson 1971) However even the lowest IVT dosage of angiotensin II previously used to demonstrate this sodium-angiotensin interaction (0.? ng/kg min) (Olsson and Kolmodin 1974) must be considered as pharmacological. The present expts show that <10% of that amount of angiotensin is needed to cause a marked strengthening of the dipsogenic and antidiuretic effects of IVT infusions of hypertonic NaCl No data are available regarding the physiological range of CSF angiotensin concentration in the goat However it seems unlikely that the present infusion of 25 pg/kg min of angiotensin II into the lateral ventricle raised CSF concentration of the octapeptide several times above the extreme level (12 ng/100 ml) which Abraham *et al* (1975) observed in the sodium depleted sheep Lower IVT dosages of angiotensin II than the present have not been tested so far but it is hoped that further investigations of cerebral sodium-angiotensin interaction may help to provide a definite answer to the question whether activation of the renin-angiotensin system plays a physiological role in the regulation of thirst and ADH release.

This work was supported by the Swedish Medical Research Council (project 04X-00503) and by grants from Karolinska Institutet. The generous gift of Hypertensin from the Hässle–Ciba–Geigy Company is gratefully acknowledged.

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In general, a succinct style and restriction to the necessary of documentation and discussion effectively aids in reducing publication time.

References should be given with full title and name of journals, abbreviated in accordance with 4th Ed. of *World List of Scientific Periodicals*, with volume number and first and last page numbers.

Figures should not be larger than manuscript pages and sent in as glossy prints in a size larger than that required for reproduction. Lettering should be large enough to permit suitable reduction and preferably of uniform size. When possible, diagrams and photomicrographs should extend horizontally rather than vertically in order to save space. Photomicrographs should be calibrated on the print (not as enlargement factor in figure text). Figure texts should be assembled on separate sheets.

Tables should be kept at minimum, both in number and size, with text above the table (not on separate sheets). Single numbers in a series should be replaced by mean and S.D. or mean and S.E., in the latter case with number of observations.

Key words (5–10) are recommended in order to facilitate indexing.

For abbreviations, units, and symbols see special list in the Journal and recent articles.

More detailed instructions to authors are under preparation, pending the recommendations of The Scandinavian Publications Committee.

The international system of units (SI)

The following symbols and units, recommended by the *SI* are being used in *Acta Physiologica Scandinavica*. Certain units, not included in SI will still be permitted.

SI units with recommended symbols

Units	*Symbols*
kilogramme	kg
second, millisecond	s ms
mole, millimole, micromole, nanomole, picomole	mol mmol μmol nmol pmol
meter millimeter micrometer nanometer	m mm μm nm
candela	cd
steradian	sr
hertz (frequency)	Hz (s^{-1})
newton (force)	N (kg m/s^2)
pascal (pressure)	Pa (N/m^2)
joule (energy)	J (N m)
watt (effect)	W (J/s)
lumen (lightflow)	lm (cd sr)
lux (illumination)	lx (lm/m^2)

Conversion factors to be given in Methods.

Permitted non-SI units

Units	*Symbols*
gramme	g
minute	min
hour	h
molarity (mol/liter)	M
(calorie)	cal (4.184 J)
(kilopond)	kp (9.81 N)
(millimeters of mercury)	mm Hg (1.333 bar)
(millibar)	mbar (100 Pa)
curie	Ci
liter milliliter microliter	l ml μl
degree Celsius	°C